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Office des recherches sur les pêcheries du Canada
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Associations and Species Diversity in Benthic Macroinvertebrates of Bay of Quinte and Lake Ontario[1]

M. G. JOHNSON

Department of Zoology
University of Guelph, Guelph, Ont.

AND R. O. BRINKHURST

Department of Zoology
University of Toronto, Toronto, Ont.

JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Associations and species diversity in benthic macroinvertebrates of Bay of Quinte and Lake Ontario. J. Fish. Res. Bd. Canada 28: 1683–1697.

Three main macroinvertebrate associations were found in the Bay of Quinte, Prince Edward Bay, and the adjacent area of Lake Ontario: a chironomid association of the eutrophic inner and middle bays; an association of many species of sphaeriids, oligochaetes, chironomids, and crustaceans in the mesotrophic lower Bay of Quinte and Prince Edward Bay; and a cold-stenotherm association of the oligotrophic deep basin of Lake Ontario. An additional, but more confined (inner Prince Edward Bay), association consisted of a variety of taxa, whose distribution appeared to be related to the distribution of vascular vegetation. All of the main taxonomic groups contributed to each association and to differences among associations. The lakeward progression of associations in the study area was closely similar to the west to east shifts in Lake Erie reported earlier. Species diversity in Lake Ontario associations apparently was related to degree of eutrophy and to water temperature.

JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Associations and species diversity in benthic macroinvertebrates of Bay of Quinte and Lake Ontario. J. Fish. Res. Bd. Canada 28: 1683–1697.

Nous avons découvert trois associations majeures de macroinvertébrés dans la baie de Quinte, la baie du Prince-Edouard, et le secteur avoisinant du lac Ontario: une association de chironomidés dans les eaux eutrophes des zones intérieures et intermédiaires des baies; une association de plusieurs espèces de sphaeriidés, oligochètes, chironomidés et crustacés dans la partie inférieure mésotrophe de la baie de Quinte et de la baie du Prince-Edouard; et une association sténotherme froide dans le bassin profond oligotrophe du lac Ontario. Nous avons

[1]This paper is the first in a series of three on the benthos of the Bay of Quinte and the adjoining part of Lake Ontario.

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observé une association additionelle confinée à l'intérieur de la baie de Quinte et comprenant une variété de taxa dont la distribution semble liée à celle des plantes vasculaires. Chaque association comprend des représentants de tous les grands groupes taxonomiques qui contribuent également à différencier les associations entre elles. La progression vers le lac des associations de la région sous étude est semblable aux déplacements d'ouest en est signalés antérieurement pour le lac Erié. La diversité des espèces caractéristiques des associations du lac Ontario semble dépendre du degré d'eutrophie et de la température de l'eau.

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THE distribution and relative abundance of many benthic macroinvertebrates may be expected to reflect some general level of production, or trophic status, because the very processes of production directly alter the benthic environment. This concept motivated studies in lake typology and made so-called indicator species popular among some pollution biologists. Such classifications have been criticized for their lack of applicability and their rigidity (Hynes 1960). Similarly, subjective categorization of macroinvertebrate associations in relation to trophic status may be criticized. Yet numerous studies on benthic associations of the St. Lawrence Great Lakes have pointed to similarities in their specific composition in relation to apparent trophic state (Brinkhurst 1967; Brinkhurst et al. 1968; Johnson and Matheson 1968; Hiltunen 1967, 1969; and others).

Our objective in the present study (part I) was to determine the number and character of macroinvertebrate associations in the Bay of Quinte and the adjacent area of Lake Ontario, where variety in ecological conditions ranged from gross pollution in the inner Bay of Quinte to depth-dependent oligotrophy in open Lake Ontario.

We attempted to establish the degree of difference between the associations and to determine which of the main taxonomic groups contributed towards these differences. The respective positions in the trophic gradient of these associations were compared with those of associations described elsewhere in the St. Lawrence Great Lakes. Finally, we examined species diversity in these macroinvertebrate associations for its potential value in describing the trophic status of the community. Due regard was given possible confounding factors such as the constraining influence of low temperature on species diversity.

The significance of cultural eutrophication in the Bay of Quinte was described by McCombie (1966 and 1967) and Johnson and Owen (1971); these papers adequately describe this part of the study area. Changes in Lake Ontario have been documented by Beeton (1966), Schenk and Thompson (1965), and others. These reports and those of Anderson et al. (1961), and Brinkhurst et al. (1968), Dobson (1967), Hiltunen (1969), Johnson and Matheson (1968), Rodgers and Anderson (1963), and Scott and Lansing (1967) include background limnological data on Lake Ontario.

Methods

COLLECTION AND IDENTIFICATION OF MACROINVERTEBRATES

Extensive sampling was conducted throughout the bay and in the adjacent area of the lake to determine species distributions and diversity and to delineate associations. Intensive sampling was conducted at four stations with fauna representative of broad subdivisions of the study area to provide data on temporal variations in composition of associations and in species diversity.

Extensive sampling was carried out from May to early June of 1966 along 33 transects between Trenton and the bay mouth (Fig. 1). Additional sampling was carried out in the summers of 1967 and 1968, when samples were taken along 11 transects in Prince Edward Bay and Lake Ontario. A single set of five or six samples was taken along each transect. Transects were selected with little prior knowledge of the limnology of the area, other than that it showed a gradient in trophic character, and with no knowledge of the benthos.

Samples were obtained with a slightly modified 22.5 × 22.5-cm Ekman grab. The suspension frame was removed and replaced with one with longer arms, which allowed the upper doors to open to nearly the full width of the grab to reduce the shock wave transmitted in descent. The grab performed well in the substrates normally sampled, penetrating 15–20 cm with minimal disturbance of the mud–water interface. The only difficulty encountered was in taking seven littoral samples in Prince Edward Bay, where vascular plants interfered, and in taking about the same number of samples in hard sandy substrates throughout the study area. Samples were emptied into a large tub and washed in a screen-pail with 0.6-mm apertures. The live animals were removed, usually within several hours, and preserved in 70% ethyl alcohol.

Intensive sampling at the four stations (Big Bay, Glenora, Conway, and Lake Ontario) was carried out monthly between April 1967 and September 1968. Six samples were taken on each visit, although on a few occasions this objective was not met for various reasons, notably weather. Each sample was handled as described above. Each dredge sample, minus its cored subsample, represented 0.05 m^2.

Additional data, from several samples of benthos taken from the central area of Lake Ontario in 1967, were made available by Mr W. Kinney, USA Federal

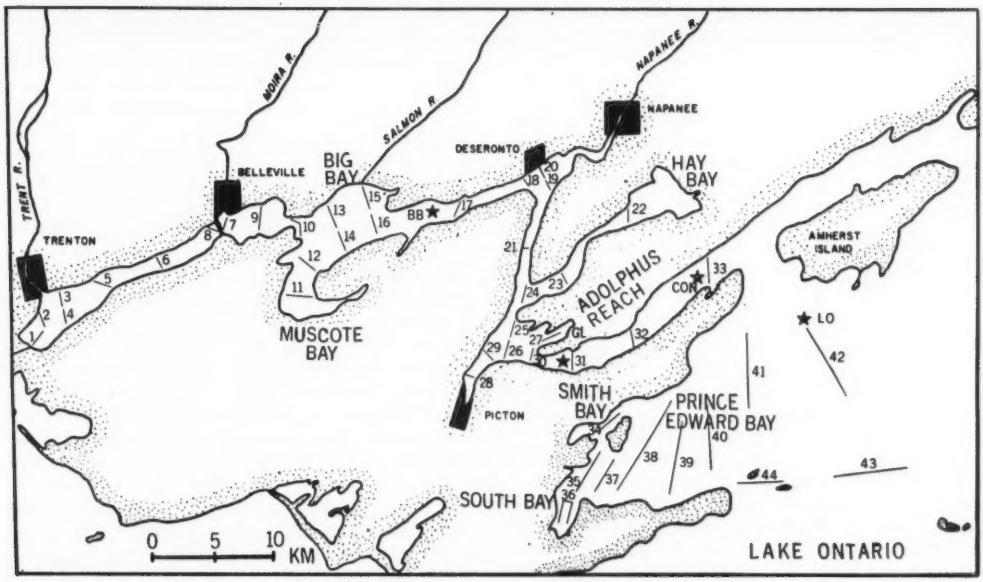

FIG. 1. Quinte study area showing stations used in the extensive collecting of macroinvertebrates (numerals) and the main stations used in intensive collecting (BB, Big Bay; GL, Glenora; CON, Conway; LO, Lake Ontario). The source of the FWPCA samples, near the middle of Lake Ontario, is not shown.

Water Pollution Control Administration (FWPCA), who used a Ponar grab and U.S. Standard No. 30 (0.6-mm aperture) screen.

Oligochaetes were mounted in Amman's lactophenol or polyvinyl lactophenol and identified using Brinkhurst's (1964, 1965) descriptions and keys. All oligochaetes taken in the extensive sampling were examined, and up to about 35 from each of the usual six samples taken at the four main stations on each visit; that is, about 200 per set.

H. B. Herrington examined all sphaeriids taken in the extensive collecting and checked about one-sixth of the collections from the main station. Nomenclature is that of Herrington (1962, 1965).

Chironomids were examined first with a dissecting microscope and divided into groups of apparent specific rank. Representatives of each group were mounted in polyvinyl lactophenol. Revisions in assignment were made after identification of these and additional mounted specimens. Selected specimens were reared to obtain larval and pupal exuviae and imagoes to confirm identification of the more common species. Nomenclature in almost all instances is that of Sublette and Sublette (1965).

DELINEATION OF COMMUNITIES

Two measures were used to delineate communities, the *coefficient of community* and *percentage similarity of community* (Jaccard 1932; Renkonen 1949; Kontkanen 1957; Whittaker 1952; Whittaker and Fairbanks 1958; Sanders 1960). The coefficient of community measures the percentage of species shared by two samples as:

$$CC = \frac{c}{a+b-c} \cdot 100,$$

in which a is the number of species in the first sample, b is the number of species in the second, and c is the number of species occurring in both. The relative abundance of species, not considered in the coefficient of community, is included in the percentage similarity of community $PS_C = 100 - 0.5 \sum |a' - b'| = \sum \min (a', b')$ in which a' and b' are, for each species, the respective percentages of the total animals in samples A and B. Both indices take values between 0 and 100, which represent the lowest and highest affinity respectively between two samples.

As pointed out by Whittaker and Fairbanks (1958), each measurement has its advantages and limitations. The coefficient of community over-values rarer species, which the percentage similarity of community neglects. Used together in the present study the two indices are useful in determining whether high affinity of samples is due not only to the sharing of most species but also to the occurrence of these species in about the same proportions.

Both indices were computed for all comparisons among the 44 sets of samples from the extensive sampling.

Similarly, the monthly sets of samples for each of the four main stations were compared with one another to determine the magnitude of each index that could be taken as indicative of high, intermediate, or low affinity. Comparisons among all months at each station showed how similar the composition of the community remained over a period of time, that is, how well the community maintained its structure temporally.

Diversity

The entropy formula, as applied to information analysis,

$$\bar{H} = -k \sum_{i=1}^{m} p_i \log p_i$$

was used to calculate diversity per individual (Margalef 1957; MacArthur and MacArthur 1961; Patten 1962), where m species have probabilities of occurrence p_i and $\bar{H}$ is the mean diversity (or information) per individual. Base 2 logarithms were used and, with $k = 1$, $\bar{H}$ is expressed as bits (binary digits).

Diversity per individual was calculated for all extensive and intensive sets of five or six samples from the study area. Diversity in macroinvertebrate associations in Hamilton Bay and the adjacent area of Lake Ontario was calculated from the data of Johnson and Matheson (1968) and of Hiltunen (1969).

Distribution of Macroinvertebrates

A total of 134 taxa, most of which were identified to species, was collected. There were 20 species of oligochaetes, 22 sphaeriids, 43 chironomids, and 6 crustaceans. Although representatives of these four groups comprised only two-thirds of the number of species, they accounted for 96.5% of the total number of animals collected. The following account of the distribution of macroinvertebrates concerns mainly the common species in these four groups; however, an annotated tabulation of all taxa is included (Appendix 1).

Relatively ubiquitous species included the oligochaetes *Limnodrilus hoffmeisteri* and *Ilyodrilus templetoni*, the sphaeriid *Pisidium casertanum*, the chironomids *Chironomus plumosus* and *C. digitatus*, and the amphipod *Gammarus fasciatus*.

Two species groups, *Chironomus attenuatus* gr. and *Procladius denticulatus* gr., were known to contain at least two species on the basis of reared adults. *Chironomus attenuatus* gr. contained *C. anthracinus* as well as *C. attenuatus*; the former probably was the more common species of the two in the outer bay but was not obtained in reared specimens from the inner and middle bays. Therefore, only *C. attenuatus* should be considered widespread. Similarly, *P. denticulatus* gr. consisted of *P. freemani* and *P. denticulatus* at least. Only the latter was reared from lower Adolphus Reach and from Lake Ontario, indicating that *P. freemani* was probably restricted mainly to the inner and middle bays; *P. denticulatus* apparently was widespread.

The distributions of an additional 27 relatively common species are shown in Fig. 2. These species comprised 91% of the animals collected in extensive sampling including the designated ubiquitous species.

Three main groupings of chironomids were apparent (Fig. 2). The species *Coelotanypus concinnus*, *Procladius adumbratus*, *Chironomus amachaerus*, *Tanytarsus* sp. A, and *Tanytarsus* sp. B. occurred in the inner and middle bays. The species *Chironomus atritibia*, *Tanytarsus* sp. C, and *Micropsectra* nr. *dives* occurred in Adolphus Reach and Prince Edward Bay. The only species of chironomid in the deepwater lake samples was *Heterotrissocladius subpilosus*.

A similar grouping was found in the oligochaetes. *Peloscolex multisetosus* was confined to the inner and middle bays, those taken in upper Adolphus Reach being *P. multisetosus* form *longidentus*. A group consisting of *Peloscolex ferox*, *Aulodrilus pluriseta*, *Potamothrix bavaricus*, *P. vejdovski*, and *P. moldaviensis* occurred in Adolphus Reach and most of Prince Edward Bay. *Stylodrilus heringianus* was found only in samples from deep areas of Lake Ontario and from stations 39, 42, and, most abundantly, 43. However, *Tubifex tubifex* occurred in both the second and third groups, that is, in Adolphus Reach and the deeper areas of Prince Edward Bay and in the FWPCA deepwater samples from Lake Ontario.

The distribution of the common sphaeriids paralleled those of chironomids and oligochaetes. Two species, *Pisidium henslowanum* and *P. subtruncatum*, occurred in the middle bay, Adolphus Reach, and Prince Edward Bay. (Only two and three individuals of the former and latter species respectively were collected in the inner bay, so they should not be considered widespread.) Four other species, *P. lilljeborgi*, *P. idahoense*, *P. ventricosum*, and *Sphaerium corneum*, were taken in Adolphus Reach and Prince Edward Bay. *Pisidium conventus* and *Sphaerium nitidum* were taken only in Prince Edward Bay and adjacent lake stations, but, of these two, only *P. conventus* occurred in the FWPCA deepwater samples.

The isopod *Asellus racovitzai* was taken in significant numbers only in Adolphus Reach and Prince Edward Bay. It was not present in deepwater samples from the lake and was relatively rare or absent in samples from stations 41–43. In contrast, *Ponto-*

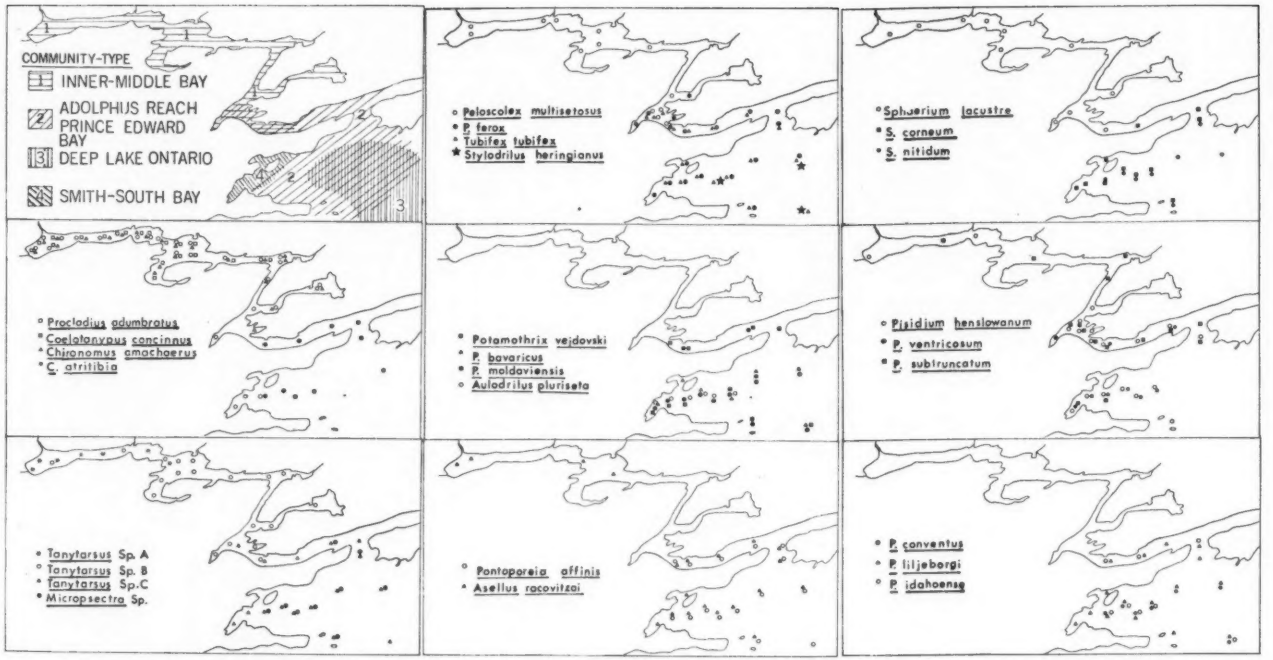

FIG. 2. Distributions of the common macroinvertebrates of the study area. The several ubiquitous species are not included. The upper-left panel shows the associations determined from mathematical analysis described in text.

poreia affinis was common only at stations 33 and stations 37–44 and it extended into the Bay of Quinte no farther than station 31. It was dominant in FWPCA samples.

Delineation of Associations

Results from the main stations are presented first to provide an objective method of interpretation of the values of the two indices. Fifteen sets of samples were taken at the Big Bay, Glenora, and Conway stations through the study period. Ten sets were taken at the Lake Ontario station. The averages for all comparisons (each set compared with all other sets) for each index are provided in Table 1.

Comparison of consecutive sets was expected to provide the most precise criteria for assessing affinity. The average values of the two indices based on consecutive sets (Table 1) were not much greater than those that included all comparisons, which indicates consistency in composition of the macroinvertebrate fauna throughout the study period.

Finally, comparisons made among sets that were taken in the same month 1 year apart are of interest, because, if affinity happened to be generally low, instability characteristic of changing successional associations would be indicated. However, no differences of any apparent importance were evident between the values obtained from consecutive months and values from the same months 1 year apart. Stability was evident in the composition of the macroinvertebrate associations at the main stations throughout the study.

The data from the main stations are of value in setting up criteria to assess degrees of affinity for the two indices. Based on the foregoing analysis, a value of CC of 50 or more and a value of PS_C of 30 or more apparently indicated high affinity (the values of the two indices *within stations* did not fall below 50 for CC or 30 for PS_C). Values of one-half of these, that is, a CC of 25 and a PS_C of 15, were taken arbitrarily as separating sets with low and intermediate affinity. Analysis of the 44 sets of data from extensive sampling (Fig. 3) yielded the following observations.

Most of the comparisons of stations 1 to 29 indicated intermediate or high affinity. Of the 406 comparisons within sets 1–29, 113 had CC values that exceeded 50 and only 48 had CC values less than 25 and practically all of these involved stations 25–29. Of 406 comparisons of PS_C, 140 had values over 30 and only 35 values less than 15. Stations in the series 30–44 generally showed low affinity to stations in the series 1–29. Of 435 comparisons between stations in the two series, only 41 values of CC exceeded 25 and none exceeded 50. Furthermore, the majority of the 41 comparisons indicating moderate affinity involved stations 27–29 and 30–32, that is, geographically adjacent groups. The pattern in PS_C was similar, with only 42 values greater than 15, of which none exceeded 30. The majority of the comparisons that showed intermediate affinity involved stations 31–32.

Low and intermediate affinity among stations 30–44 was indicated. Of 105 comparisons, only 17 values of CC and 14 of PS_C showed high affinity. Most of the high-affinity values were within stations 37–40 plus 44 and within stations 41–43. None of the stations 34, 35, and 36 had high affinity with any other station in the entire series.

The above analysis showed, as indicated in Fig. 2, that the macroinvertebrate fauna in the Bay of Quinte was homogeneous between Trenton and Picton with some departure at the latter location. A second association occurred below Glenora and extended into Prince Edward Bay and had some resemblance to the first, or inner-bay, association in upper Adolphus Reach. Intergradation of the two associations between Picton Bay and middle Adolphus Reach was shown by the analysis. A third association was indicated near the mouth of Prince Edward Bay and in Lake Ontario. According to

TABLE 1. Average values, determined in three ways on data from intensive sampling at the four main stations, of the two indices *coefficient of community* (CC) and *percentage similarity of community* (PS_C) as described in text.

	All possible comparisons		Comparisons of consecutive sets		Comparisons same months 1 year apart	
Station	CC	PS_C	CC	PS_C	CC	PS_C
Big Bay	53	30	61	32	56	33
Glenora	63	34	66	36	65	34
Conway	66	31	70	38	67	40
L. Ontario	55	38	58	41	55	38
Avg	59	33	64	37	61	36

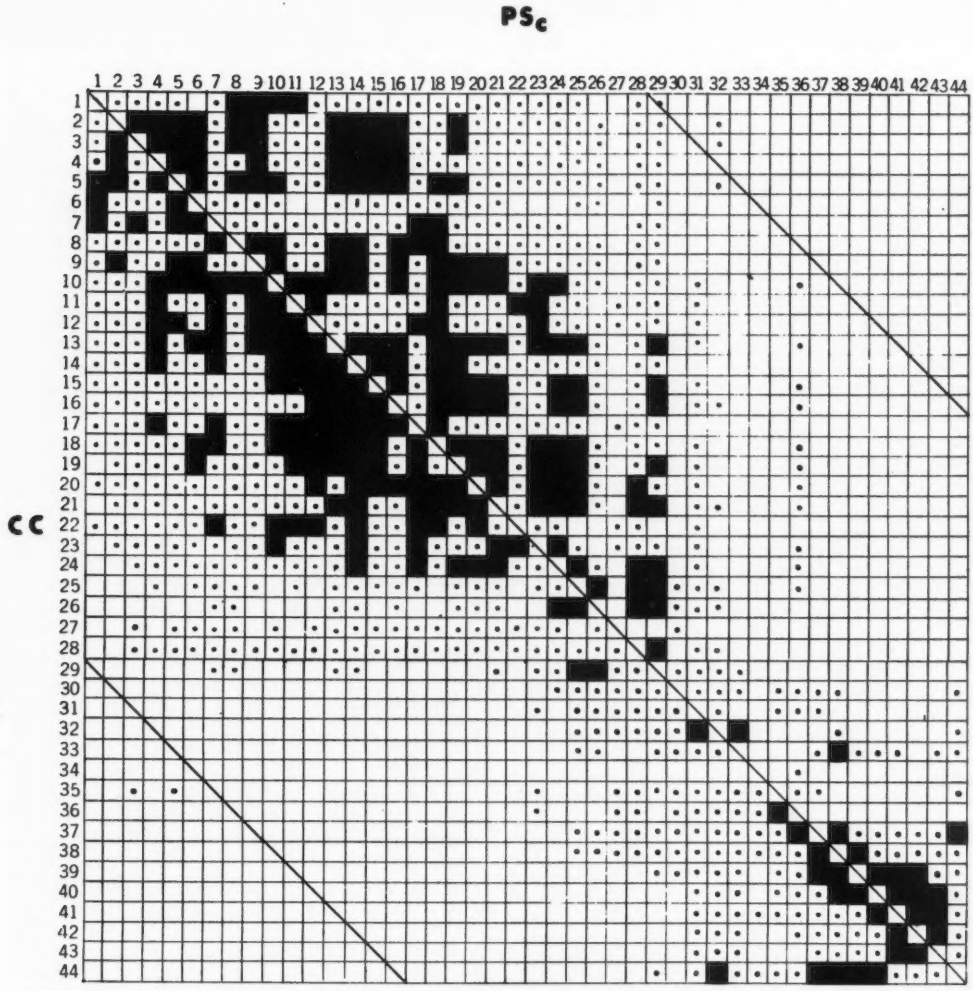

FIG. 3. Values of the coefficient of community (CC, lower triangle) and percentage similarity of community (PS_C, upper triangle) for stations 1–44 (Fig. 1) in the extensive collecting. Solid squares indicate high affinity between the macroinvertebrate associations of two stations; dots, intermediate affinity; open squares, little if any affinity.

comparisons among stations 41–43 and the FWPCA set of samples from the deep basin of Lake Ontario, station 43 was like the deepwater association (PS_C was 72), but stations 41 and 42 were probably transitional between those in deepwater Lake Ontario and in lower Adolphus Reach and Prince Edward Bay. The macroinvertebrate fauna of South Bay and Smith Bay possibly comprised another association; although heterogeneous, it was more like the first than like the second.

Diversity

Calculated diversity ($\bar{H}$) of each set of samples taken in extensive collecting (Fig. 4) showed considerable variation. In general, diversity was intermediate but rather static between Trenton and Glenora, increased in upper Adolphus Reach (a transitional zone between two associations), and decreased lakeward to a minimum level characteristic of the deepwater association. Intermediate values

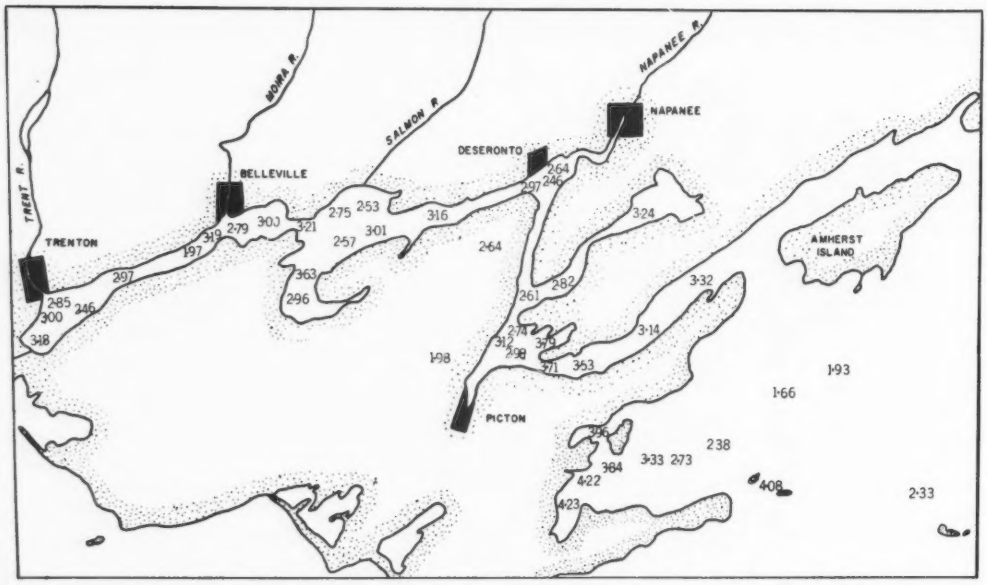

FIG. 4. Values of diversity per individual ($\overline{H}$) in collections made at the 44 extensive-sampling stations.

occurred through the inner and middle arms of the Bay of Quinte with high values locally, for example, near the mouth of the Trent River, in Muscote Bay, and in Hay Bay. Generally, lower values were found in the "mainstream" basins and reaches of the inner and middle bays.

High values were found in Adolphus Reach and in much of Prince Edward Bay. However, sampling in Adolphus Reach near the bay mouth in the spring of 1966 provided samples with higher diversity than average because the dominant species, *Pontoporeia affinis*, occurred in lowest numbers in the spring. The highest values of $\overline{H}$ were found in Smith Bay and South Bay and the lowest in the lakeward stations 41–43. Deepwater samples from Lake Ontario had a low diversity per individual of 1.77.

The diversity values for the macroinvertebrates obtained by intensive sampling at the four main stations were as follows:

	Mean	Range
Big Bay	2.99	2.58–3.34
Glenora	3.14	2.59–3.66
Conway	2.26	1.00–3.26
L. Ontario	2.04	1.60–3.15

Results from Big Bay and Glenora were in general agreement with the data from extensive sampling. The wide range in diversity at Conway was related to the life cycle of *P. affinis*. The average diversity at Conway was much lower than springtime values, which explains the lack of agreement with the 1966 May–June data from station 33. The range in $\overline{H}$ was narrower at the lake station than at Conway probably because most, if not all, *P. affinis* reproduce at the age of 2 years in the lake and their numbers fluctuate seasonally less than at Conway.

Discussion

The extensive areas of intergradation of the macroinvertebrate associations is to be expected in the gradual eutrophic–oligotrophic gradient of the study area. Intergradation may have obscured boundaries but not the presence of several associations. For example, the second association (in Adolphus Reach and Prince Edward Bay) is not a blend of the first (inner bay) and third (deepwater Lake Ontario) associations because it contained numerous species not found in the other two associations. The macroinvertebrates found in samples from Smith Bay and South Bay probably represent an assemblage transitional between the second association and a distinct littoral association apparently dependent on the presence of vascular vegetation. Several species, notably chironomids (*Chironomus abortivus*, *C. subtendens*, *Cricotopus* sp., *Psectrocladius* spp.) and miscellaneous taxa

(for example, the isopod *Lirceus lineatus*, the amphipod *Hyalella azteca*, the mayfly *Caenis* sp. and the lepidopteran *Synclitus* sp.) were found in western Prince Edward Bay in the extensive sampling but only in areas with vascular vegetation.

Species from all of the main groups (oligochaetes, sphaeriids, chironomids, and crustaceans) contributed to differences among associations. Clearly, there is no justification for selection of any one group for study over another if differences in associations are to be described adequately.

Consistency in relative abundance of component species was evident within associations. That is, the species occurred in approximately the same proportions at stations within each association as shown by the close relation between the two indices (Fig. 3).

The correspondence of the sequence of associations along the Trenton–Lake Ontario transect with associations along the west–east transect in Lake Erie is rather striking. *Limnodrilus* spp., *Chironomus plumosus*, *Procladius* spp., and *Coelotanypus* sp. are abundant in western Lake Erie (Brinkhurst et al. 1968) and in the inner Bay of Quinte. This association is replaced toward the central basin of Lake Erie by one containing *Peloscolex ferox*, *Potamothrix* spp., *Aulodrilus* spp. and a greater variety of sphaeriids than occurs either eastward or westward. In fact, the association of the central basin of Lake Erie closely resembles that in Adolphus Reach and Prince Edward Bay. The benthos of the eastern basin of Lake Erie resembles that of the main basin of Lake Ontario. *Pontoporeia affinis*, *Stylodrilus heringianus*, and *Tubifex tubifex* are abundant in both of these basins. *Chironomus anthracinus*, *C. attenuatus*, and *Heterotrissocladius subpilosus* are common in both basins and the latter apparently is confined in Lake Erie to the eastern basin.

The so-called cold-stenotherm or deepwater association (Fig. 2) of *P. affinis*, *Pisidium conventus*, *S. heringianus*, and *H. subpilosus* is found in deep, well-oxygenated, offshore sediments and, therefore, it presently occurs over more of the area of the Great Lakes than any other assemblage of species (Brinkhurst et al. 1968). The cosmopolitan *Limnodrilus hoffmeisteri* and peculiarly distributed *T. tubifex* (in both polluted and oligotrophic basins) are common in this association, and several rare tubificids may be collected, depending mainly on sampling intensity. These are *Aulodrilus americanus*, *Peloscolex variegatus*, *Rhyacodrilus montana*, *R. sodalis*, and *R. coccineus*, which were not observed in the present study, but which were reported (Brinkhurst et al. 1968) in Lake Ontario previously.

The *T. tubifex–Limnodrilus* spp. association (*L. cervix*, *L. claparedeanus*, *L. undekemianus*, *L. hoffmeisteri*, and, in Lake Erie, *L. maumeensis*) is characteristic of the profundal of polluted harbours and near river mouths (Brinkhurst 1967, 1970; Johnson and Matheson 1968; Hiltunen 1967). Apparently this association is the eutrophic counterpart of the oligotrophic cold-stenotherm association. Evidence from deep basins to date, for example from southern Lake Michigan and central Lake Erie (Hiltunen 1967; Brinkhurst et al. 1968), indicates that these associations are changing toward the *T. tubifex–Limnodrilus* spp. type. Also, *Potamothrix* spp. and *Aulodrilus* spp. apparently occupy an intermediate position in this progression, perhaps being indicative of mesotrophic conditions. Prince Edward Bay, with relatively abundant *Potamothrix* spp. and *Aulodrilus* spp., appears to be in this mesotrophic position. The *T. tubifex–Limnodrilus* spp. association was not found there, but most of the component species were present (Appendix 1).

Although a variety of factors may influence species diversity, temperature and position in the trophic gradient (the latter integrates several factors) appeared to be of most general importance. Low temperature apparently inhibits diversity in Lake Ontario, although depth and pressure also may be significant. Temperatures over the sediments averaged 11 C at the station in the inner bay, 8 and 7 C in upper and lower Adolphus Reach, 6 C in the main basin of Prince Edward Bay, and 3.5 C in Lake Ontario; and the maximum temperatures were 22, 20, 18, 17, and 4 C respectively (Johnson and Brinkhurst 1971).

Low species diversity ($\overline{H}$ between 1.0 and 2.0) in the oligotrophic deep-basin association (Table 2) results in part from the lack of diversity in sphaeriids and chironomids and their small populations relative to numbers of *P. affinis*. In the deep eutrophic basins, species diversity declines ($\overline{H}$ below 1.0) but is maintained at a low level by a few species of eurytopic oligochaetes. Finally, diversity may approach and even attain zero in local areas, such as the 2 km^2 area in Hamilton Bay where Johnson and Matheson (1968) were unable to obtain any macroinvertebrates. Chironomids have minimal informative value in the transition from oligotrophy to eutrophy in deep cold (3–4 C) basins because of their scarcity. At slightly higher temperatures (5–6 C) *Procladius denticulatus* can be expected to increase in response to an increase in production of oligochaetes, unless conditions are extremely adverse as shown by the lack of *Procladius* in the main basin of Hamilton Bay.

TABLE 2. Species diversity in benthic macroinvertebrate associations in three broad divisions of habitat in Lake Ontario.

Habitat	Location	Species diversity ($\bar{H}$)	Source of data
Cold deep basins	FWPCA samples, L. Ontario	1.77	Present study
	L. Ontario, >100 m deep	1.20	Hiltumen 1969
	L. Ontario, western basin	1.50	Johnson and Matheson 1968
Basins of intermediate depth and temperature	L. Ontario station	2.04	Present study
	Conway station	2.26	" "
	Glenora station	3.14	" "
	Hamilton Bay	0.89	Johnson and Matheson 1968.
Warm, shallow, inshore waters	Big Bay station	2.99	Present study
	Inner Prince Edward Bay	4.10	Present study
	Hamilton Bay	1.80	Johnson and Matheson 1968

A wide range in trophic conditions may be observed in warm shallow waters of the Great Lakes. Their relation with the benthic macroinvertebrate community is, however, obscured by hydrologic factors, particularly as these determine the character of substrate. Shallow areas with strong currents are relatively free of macroinvertebrates under oligotrophic conditions, but under eutrophic conditions large numbers of amphipods and chironomids (*Gammarus fasciatus* and *Cricotopus* spp. mainly) are associated with the green alga *Cladophora* on bedrock and boulders, (Ontario Water Resources Commission unpublished data). The change in species composition with increased eutrophy in areas of active sediment deposition may be inferred by comparisons among associations of inner Prince Edward Bay, the inner Bay of Quinte, and the littoral–sublittoral zone of Hamilton Bay. Inner Prince Edward Bay contained a high diversity ($\bar{H}$ of 4.10) association of many species of chironomids (*Cricotopus*, *Trissocladius* spp., and other orthoclads, with *Chironomus subtendens* and *C. abortivus*) together with a variety of species from many groups (Appendix). The association of the inner Bay of Quinte was dominated by *Chironomus plumosus*, *C. attenuatus*, and *Procladius* spp. and showed less diversity ($\bar{H}$ of about 3.00). Finally, the inshore sediments of Hamilton Bay contained a small number of oligochaete species and diversity was low ($\bar{H}$ of 1.80).

Miller (1941) inferred from his studies on chironomids in an Ontario lake that the higher temperature of shallow waters encouraged the development of species diversity. However, the structural diversity provided by vascular plants (or the simulation of varied stream conditions on a stony shore) must be at least partly responsible. That is, the littoral environment apparently is more "coarse-grained" in the MacArthur sense (MacArthur and Connell 1966) than the deeper lake environment. The decrease in diversity in the macroinvertebrate community from mesotrophy through eutrophy, indicated by the comparison of inner Prince Edward Bay with the inner Bay of Quinte, may be due partly to the replacement of vascular plants by phytoplankton blooms of mainly blue-green algae in eutrophic shallow water.

The interaction of temperature and position in the trophic gradient in influencing species diversity is generally apparent. Diversity over much of the trophic range at high temperature exceeded diversity over a wide trophic range at low temperature but the overlap in the ranges of diversity was slight. In fact, the diversity of the cold-water oligotrophic association of Lake Ontario was the same as that of the warmwater eutrophic association of Hamilton Bay. Temperature should be recognized as a factor that can influence diversity independently of or together with trophic status. The modes of action of cold and cultural eutrophication are similar, each

being an overriding factor that reduces diversity of niches and, hence, reduces the fauna to a very few of the minority of specialized and eurytopic species.

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Appendix I

The following species were taken in extensive and intensive sampling during the period 1966–68 in the Bay of Quinte and the adjacent part of Lake Ontario. Complete names are presented here with brief notations of distribution and abundance *in samples*.

Oligochaetes	
Tubificidae	
Tubifex tubifex (Müller)	Ad., Rch.[1], lake; abundant
Ilyodrilus templetoni (Southern)	Widespread; common
Potamothrix bavaricus (Oschmann)	Ad. Rch., lake; common
P. vejdovski (Hrabě)	" " "
P. moldaviensis Vejd. and Mrazek	" " "
Limnodrilus hoffmeisteri Clap.	Widespread; abundant
L. udekemianus Clap.	Lake; one record
L. cervix Brinkhurst	Inner bay; rare
L. claparedeanus Ratzel	Lake; rare
L. profundicola (Verrill)	Lake; one record
Peloscolex ferox (Eison)	Picton Bay, Ad. Rch., lake; abundant
P. multisetosus (Smith)	Inner bay; common
P. freyi Brinkhurst	P.E. Bay;[1] rare
Aulodrilus limnobius Bretscher	Scattered records; rare
A. pluriseta (Piguet)	Ad. Rch., P.E. Bay; common
A. pigueti Kowalewski	Big Bay, Glenora; common
Lumbriculidae	
Stylodrilus heringianus Clap.	Lake; abundant
Naididae	
Nais spp.	Lake; rare
Stylaria lacustris (L.)	Lake; rare
Slavina appendiculata (d'Udek.)	Conway; 1 record
Leeches	
Glossiphoniidae	
Glossiphonia complanta (L.)	P.E. Bay; rare
G. heteroclita (L.)	Smith Bay; rare
Helobdella stagnalis (L.)	Widespread; common
Flatworms	
Planariidae	
Phagocata spp.	Smith Bay; rare
Rhabdocoela	
Hydrolimax cf. *grisea* Hald.	Widespread; common
Unidentified species	Locally common
Clams	
Sphaeriidae	
Sphaerium corneum (L.)	Ad. Rch., P.E. Bay; locally abundant
S. lacustre (Müller) f. *jayense*	Inner bay; rare
S. nitidum Clessin	P.E. Bay; common
S. partumeium (Say)	Glenora; 1 record
S. striatinum (Lamarck)	Ad. Rch., P.E. Bay; locally common
S. simile (Baker)	Ad. Rch.; rare
S. transversum (Say)	Picton Bay, P.E. Bay; rare
Pisidium adamsi Prime	Ad. Rch., P.E. Bay; rare
P. amnicum (Müller)	Ad. Rch.; 1 record

[1]Ad. Rch., Adolphus Reach; P.E. Bay, Prince Edward Bay.

(*Continued*)

The following species were taken in extensive and intensive sampling during the period 1966–68 in the Bay of Quinte and the adjacent part of Lake Ontario. Complete names are presented here with brief notations of distribution and abundance *in samples*.

P. casertanum (Poli)	Widespread; common
P. compressum Prime	Widespread; common
P. conventus Clessin	Lake; abundant
P. ferrugineum Prime	Ad. Rch., P.E. Bay; rare
P. henslowanum Sheppard	Ad. Rch.; abundant
P. idahoense Roper	Ad. Rch., lake; common
P. lilljeborgi Clessin	Ad. Rch., lake; abundant
P. nitidum Jenyns	Ad. Rch., P.E. Bay; common
P. punctatum Sterki	Ad. Rch., P.E. Bay; rare
P. subtruncatum Malm	Picton Bay, Ad. Rch.; common
P. variabile Prime	Ad. Rch.; common
P. ventricosum Prime	Ad. Rch., P.E. Bay; common
P. walkeri Sterki	Ad. Rch.; rare
Unionidae[2]	
Anodonta grandis Say	Widespread; locally common
Elliptio complanatus (Solander)	" " "
Lampsilis radiata radiata (Gmelin)	" " "
L. radiata siliquoidea (Barnes)	" " "
Leptodea fragilis (Raf.)	Big Bay; rare
Proptera alata (Say)	Big Bay; rare
Snails	
Valvatidae	
Valvata tricarinata Say	Picton Bay, Ad. Rch., lake; common
V. sincera Say	Picton Bay, Ad. Rch., lake; common
V. piscinalis Müller	Picton Bay, Ad. Rch.; rare
Amnicolidae	
Amnicola limosa (Say)	Ad. Rch., west. P.E. Bay; common
A. binneyana Hannibal	Middle bay; rare
A. lustrica Pilsbry	Quinte; rare
Bulimus tentaculatus (L.)	Widespread; abundant in littoral
Pleuroceridae	
Goniobasis livescens Menke	Picton Bay; 1 record
Lymnaeidae	
Fossaria obrussa Say	Picton Bay, Ad. Rch.; rare
Planorbidae	
Helisoma anceps Menke	Picton Bay, Ad. Rch.; rare
Promenetus exacuous (Say)	Smith Bay; 1 record
Gyralus hirsutus Gould	Smith Bay; 2 records
G. parvus Say	Smith Bay; 1 record
G. deflectus Say	Smith Bay; 1 record
Physidae	
Physa sp.	Ad. Rch., Smith Bay; rare
Crustaceans	
Gammaridae	
Gammarus fasciatus Say	Widespread; abundant
Talitridae	
Hyalella azteca (Saussure)	Widespread; locally common
Haustoriidae	
Pontoporeia affinis Lindstrom	Ad. Rch., Lake; abundant

[2]The mussels were not collected in Ekman grab samples. Rather, they were collected on a few occasions with a clam rake.

(*Continued*)

The following species were taken in extensive and intensive sampling during the period 1966–68 in the Bay of Quinte and the adjacent part of Lake Ontario. Complete names are presented here with brief notations of distribution and abundance *in samples*.

Mysidae	
Mysis relicta (Loven)	Lake; rare
Asellidae	
Asellus racovitzai (Williams)	Ad. Rch., P.E. Bay; abundant
Lirceus lineatus (Say)	Smith Bay; common
Midges	
Chironomidae	
Procladius adumbratus Joh.	Quinte to Glenora, South Bay; abundant
P. denticulatus Sublette	Widespread; abundant
P. freemani Sublette	Bay of Quinte; abundant
Coelotanypus concinnus (Coq.)	Inner bay; abundant
Clinotanypus cf. *pinguis* (Loew)	Ad. Rch., P.E. Bay; rare
Ablabesmyia americana Fittkau	Scattered; rare
Pentaneura s. lat.	Scattered; rare
Chironomus plumosus (L.)	Widespread; abundant
C. attenuatus Walker	Widespread; abundant
C. anthracinus Zett.	Ad. Rch.; common
C. atritibia (Townes)	Ad. Rch., P.E. Bay; common
C. modestus (Say)	Widespread; common
C. digitatus Malloch	Widespread; common
C. amachaerus (Townes)	Inner bay; common
C. abortivus (Malloch)	South, Smith bays; common
C. subtendens (Townes)	South, Smith bays; common
C. jucundus Walker	Widespread; rare
Chironomus sp. A	Inner bay; locally common
Chironomus sp. B	Middle bay; rare
Phaenopsectra sp.	Inner bay; rare
Paracladopelma cf. *obscura* Brundin	P.E. Bay; 1 record
Polypedilum cf. *nubeculosum* (Meig.)	Inner, middle Bay, South Bay; common
P. cf. *simulans* Townes	Widespread; common
Microtendipes pedellus (DeGeer)	Middle bay, Smith, South bays; common
Stictochironomus sp.	Middle Bay; rare
Pseudochironomus sp.	Middle Bay; 1 record
Paratendipes albimanus (Meig.)	Bay of Quinte; common
Glyptotendipes sp. A	Inner bay; rare
Glyptotendipes sp. B	Inner bay; rare
Tanytarsus sp. A	Inner bay; common
Tanytarsus sp. B	Middle bay; common
Tanytarsus sp. C	Ad. Rch., lake; abundant
Tanytarsini sp. D	Middle bay; rare
Cladotanytarsus sp.	Hay, Smith, South bays; rare
Paratanytarsus nr. *varelus* (Roback)	Smith, South bays; common
Micropsectra nr. *dives* Joh.	Ad. Rch., lake; abundant
Cricotopus nr. *sylvestris* (Fabr.)	Smith, South bays; rare
Psectrocladius sp. A	" " " "
Psectrocladius sp. B	" " " "
Psectrocladius sp. C	" " " "
?*Diplocladius* sp.	Big Bay; 2 records
?*Hydrobaenus* sp.	Glenora; 1 record
Heterotrissocladius subpilosus (Kief.)	Conway, lake; common
Ceratopogonidae	
Palpomyia spp.	Quinte; common
Chaoborinae	
Chaoborus albatus John.	Inner bay; common

(*Continued*)

The following species were taken in extensive and intensive sampling during the period 1966–68 in the Bay of Quinte and the adjacent part of Lake Ontario. Complete names are presented here with brief notations of distribution and abundance *in samples*. (*Concluded*)

Caddisflies	
Polycentropidae	
Polycentropus sp.	Inner bay; rare
Phylocentropus sp.	Smith Bay; rare
Leptoceridae	
Leptocerus americanus (Banks)	Inner bay nr. Trenton; rare
Athripsodes dilutus (Hag.)	Smith Bay; rare
Leptocella albida (Walker)	" " "
Oecetis sp. A. Ross	" " "
Oecetis sp.	Inner bay; rare
Mystacides sepulchralus (Walker)	Middle Bay, Ad. Rch; rare
Triaenodes sp.	Inner Bay, Smith Bay; rare
Sericostomatidae	
Lepidostoma sp.	Ad. Rch.; 1 record
Mites	
Arrenuridae	
Arrenurus sp.	Hay Bay; 1 record
Hygrobatidae	
Hygrobates sp.	Scattered; 3 records
Limnesiidae	
Limnesia sp.	Inner, middle bays; common
Unionicolidae	
Unionicola sp.	Widespread; common
Neumania sp.	Inner, middle bays; common
Pionidae	
Piona sp.	Widespread; common
Lebertiidae	
Lebertia sp.	Middle bay; 1 record

Miscellaneous

An aquatic lepidopteran, *Synclitus* sp., occurred in Smith Bay with the mayfly *Caenis*. The riffle beetles *Dubiraphia* sp. and *Stenelmis* sp. were collected at Big Bay.

Production of Benthic Macroinvertebrates of Bay of Quinte and Lake Ontario[1]

M. G. JOHNSON

Department of Zoology
University of Guelph, Guelph, Ont.

AND R. O. BRINKHURST

Department of Zoology
University of Toronto, Toronto, Ont.

JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Production of benthic macroinvertebrates of Bay of Quinte and Lake Ontario. J. Fish. Res. Bd. Canada 28: 1699–1714.

Production rates (P) by benthic macroinvertebrates, obtained from data on instantaneous growth rate and biomass (B), ranged from 0.26 kcal m^{-2} day^{-1} at the inner bay station to 0.80 in the outer bay, 0.19 at the bay mouth, 0.16 outside the Bay of Quinte, and 0.04 in the main basin of Lake Ontario. Respiration rates (R) of the common species were fitted to the general model $\log_e R = a_1 T + a_2 \log_e W$, where T is temperature and W is animal weight. Q_{10}'s averaged 3.5 and exponents of animal weight averaged 0.74. Growth efficiencies (production as a proportion of assimilation) declined progressively from about 0.65 at the inner bay station to 0.35 at the Lake Ontario station. Annual turnover ratios (P:B ratios) declined from 13 in the inner bay to about 1 in the deep sediments of Lake Ontario. Turnover ratio (TR) was correlated with mean annual bottom temperature (T), $TR = \frac{T^2}{10}$, and production was predicted by $P = B \frac{T^2}{10}$.

JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Production of benthic macroinvertebrates of Bay of Quinte and Lake Ontario. J. Fish. Res. Bd. Canada 28: 1699–1714.

Le taux de production (P) des macroinvertébrés benthiques, calculé à partir de données sur le taux de croissance instantané et la biomasse (B), s'échelonne entre 0.26 kcal m^{-2} $jour^{-1}$ dans la zone intérieure de la baie de Quinte et 0.80 dans la zone extérieure, 0.19 à l'entrée, 0.16 en dehors de la baie, et 0.04 dans le bassin principal du lac Ontario. Le taux respiratoire (R) des espèces communes correspond à l'équation générale $\log_e R = a_1 T + a_2 \log_e W$, dans laquelle T représente la température et W, le poids des organismes. La moyenne des Q_{10} est de 3.5 et celle des exposants du poids des organismes, de 0.74. Le rendement de croissance (rapport production-assimilation) diminue progressivement d'environ 0.65 à la station de l'intérieur de la baie à 0.35 à celle du lac Ontario. Le taux de renouvellement (rapport P:B) annuel tombe de 13 dans la zone intérieure de la baie à environ 1 dans les sédiments profonds du lac Ontario. Le taux de renouvellement (TR) est fonction de la température moyenne annuelle du fond (T), $TR = \frac{T^2}{10}$, et nous prédisons le taux de production suivant la formule: $P = B \frac{T^2}{10}$.

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BENTHIC macroinvertebrate communities of the St. Lawrence Great Lakes have been under investigation for a century (Smith 1871; Hoy 1872). Following the advice of Forbes (1925), biologists studied macroinvertebrate communities as an aid to understanding problems in production of fish. Measurement of production of macroinvertebrates was implicit in such an approach, but not one estimate of this for any part of the Great Lakes has been published.

[1]This paper is the second in a series of three on the benthos of the Bay of Quinte and the adjoining part of Lake Ontario.

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The range from gross pollution in the inner Bay of Quinte to depth-dependent oligotrophy in open Lake Ontario (McCombie 1966, 1967; Johnson and Owen 1971) represents a gradient in ecological conditions for comparative studies of macroinvertebrate production.

Macroinvertebrate associations in relation to this gradient and other factors were described by Johnson and Brinkhurst (1971a). Utilization of energy and production rate by macroinvertebrates were measured at four stations along the trophic gradient and are examined in this paper. Production rate was calculated from instantaneous growth rates and biomass; respiration was determined in laboratory experiments; assimilation was estimated as the sum of production and respiration.

Methods

The four stations (Fig. 1) represented macroinvertebrate associations or transitions between them (Johnson and Brinkhurst 1971a). The Big Bay station represented the chironomid association typical of the inner and middle bays; the Glenora station, the transition between this association and an association of a variety of sphaeriid, tubificid, chironomid, and crustacean species found at Conway and characteristic of Adolphus Reach and Prince Edward Bay; and the Lake Ontario station, the transition between the latter association and the deep-water association of Lake Ontario. Bottom samples were taken at the four stations at approximately monthly intervals from April 1967 to September 1968. Other data were obtained from the extensive sampling programme used to evaluate associations (Johnson and Brinkhurst 1971a) and from U.S. Federal Water Pollution Control Administration (FWPCA) samples taken in the central area of Lake Ontario at depths in excess of 100 m.

Bottom samples were obtained with an Ekman grab and washed in a screen-pail with 0.55-mm apertures. A 5-cm diam core was removed from the grab and preserved with formalin, and the small forms that might have been lost in routine screening were separated with a 0.15-mm aperture screen.

Temperature and dissolved oxygen over the sediment were measured weekly during the spring and summer and less frequently at other times. A galvanic probe with a thermistor (Precision Scientific Company) was used in 1966. Subsequently, a corer (Brinkhurst et al. 1969) was obtained that provided relatively undisturbed samples of mud and water. For oxygen determination, water was removed through a side-delivery tube at a point 5 cm above the mud–water interface. Dissolved oxygen was determined by the azide modification of the Winkler method.

Respiration of macroinvertebrates was determined as follows: animals taken from the main stations were held in the laboratory in water baths with native sediments,

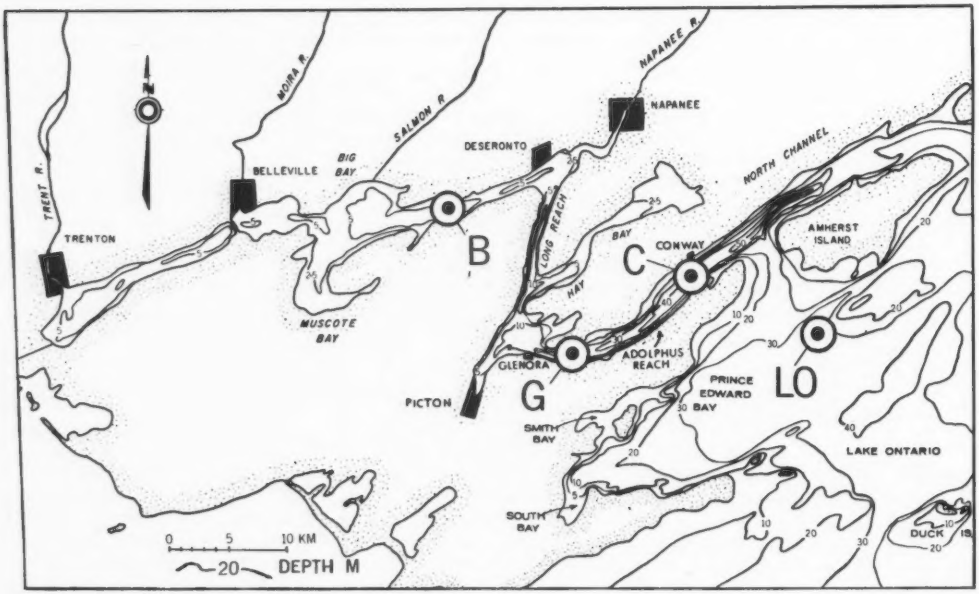

FIG. 1. The Bay of Quinte–Lake Ontario study area and the four main stations: B, Big Bay; G, Glenora; C, Conway; and LO, Lake Ontario.

and 1 day was allowed per 1 degree C shift for acclimation from ambient to test temperature. The macroinvertebrates for each analysis were sorted into groups of similar size and held without food for several hours beforehand to minimize the amount of sediment contained in their guts. About 1.5 cm of fine sand and aerated filtered water were added to 35-ml gas bottles in a controlled temperature bath, occluded air bubbles were removed, and animals were added. After 4–24 hr in darkness the concentrations of oxygen in these bottles (which decreased from 100% air saturation to not less than 50%) and others without animals were determined with a Yellow Springs Instruments Model 54 meter with BOD probe. Behaviour of the animals in the bottles appeared to be normal, that is, burrowing, tube construction, and feeding movements occurred. Animals were removed and frozen and subsequently dried, weighed, ashed at 600 C for 0.5 hr, and reweighed to determine ash-free weight. Caloric values of animals were determined with Phillipson's method (1964).

Respiration rates were calculated as $\mu g\ O_2\ mg^{-1}$ ash-free weight day^{-1} and also as $\mu g\ O_2$ per animal. An oxycaloric quotient of 0.00338 cal per mg O_2 (Ivlev 1934; Teal 1957) was used to convert to energy units. The caloric equivalent of oxygen consumption varies only 5% over the RQ range 0.71–1.00 (Slobodkin 1962), justifying the use of Ivlev's quotient.

The respiration model was of the general form

$$\log_e R = a_0 + a_1 T + a_2 \log_e W$$

(Berthet 1963; Comita 1968), where R is respiration in oxygen or calories, T is temperature, and W is individual ash-free weight. Multiple-regression analyses were made to determine the coefficients and to test the goodness of fit of the data. Oxygen concentration was not included in the model because depletion below 3 mg $liter^{-1}$ apparently did not occur at the stations.

Respiration of populations on an areal basis was calculated from mean population densities, weight frequency distributions, and field determinations of temperature.

Production was calculated as $P = G\bar{B}$, where G is the instantaneous growth rate and $\bar{B}$ is biomass or standing stock (Ricker 1946; Allen 1950; Chapman 1967). $\bar{B}$ was obtained from a series of grab samples at a particular time corresponding to a value of G on a daily basis applicable to the same time.

Values of B in cal m^{-2} for most animals were calculated from length-frequency data, weight–length equations, and caloric determinations (see Appendix 1). For oligochaetes, a weight-frequency distribution was applied directly by comparing mounted oligochaetes in regular samples with mounts from a series of preweighed worms.

Growth rates of *Pontoporeia affinis*, *Asellus racovitzai*, and several common chironomids were obtained from field data through as many intervals between sampling as possible, data being rejected for those intervals during which differential mortality, emergence, or migration were observed or suspected. As oligochaetes and sphaeriids seldom occur in recognizable cohorts in natural populations, laboratory determinations of growth rate were made as follows. Mud cores were removed from undisturbed Ekman grab samples and transferred to plastic cups 9 cm in diameter and 15 cm high. Little disturbance of the surface sediment occurred. The cores were frozen or heated (with no apparent difference between treatments noted subsequently in growth of added animals) to kill the macroinvertebrates on hand. The cores were placed in culture tanks (Fig. 2). Water dripping slowly from a supply manifold into each cup maintained the desired oxygen concentration and simulated the natural gradual renewal of water over the sediment, while most of the water was delivered to the bath to maintain the desired temperature. Groups of unidentified oligochaetes and sphaeriids obtained from field samples were blotted, weighed, and added to the cups. Although the density of animals was as much as three times field density, preliminary experiments failed to indicate any reduction in the growth rate of experimental animals at high stocking rates, and the presence of cadavers (produced by freezing or heating) did not appear to affect results either. Double and triple the weight of cadavers was added and the growth of oligochaetes was similar to growth in the standard cultures, that is, with $\times 1$ cadaver supply. The animals were removed after 25–35 days, cleaned, blotted, and weighed. If more than 20% mortality had occurred, the results were rejected.

Growth efficiency, or the proportion of assimilated energy directed to growth and reproduction, was used in the sense of "energy coefficient of growth of the second order" (Ivlev 1945), "growth efficiency" (Clarke 1946), "tissue growth efficiency" (Odum 1957), and "net efficiency of growth" (Richman 1958). This para-

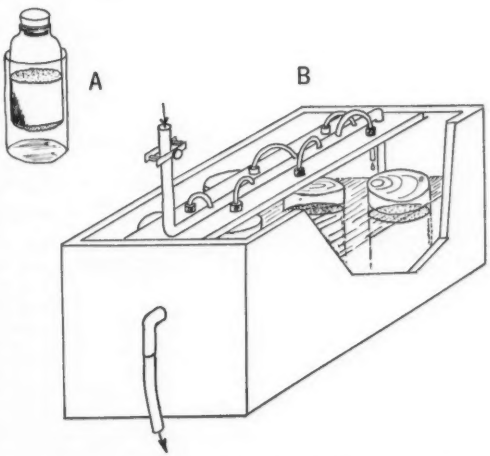

FIG. 2. A, Core sample removed to sediment container; B, water bath and sediment container used for determination of instantaneous growth rates of oligochaetes and sphaeriids. Drippers were regulated with set-screw inserts from electrical connectors.

meter was calculated for certain species, for some related groups of species, and for all benthic macroinvertebrates. Comparisons among stations were made on the basis of these calculations.

Limnology

Average bottom-water temperatures for the ice-free period in 1967 ranged from 15 C at Big Bay through 11 C at Glenora, and 8 C at Conway to 6 C at the Lake Ontario station. In 1968 these values were 1 degree C higher (2 degrees C higher at Conway). Maximum bottom temperatures (Fig. 3) showed the same trend, but were attained later in the season at the deeper than at the shallower stations. The temperature of bottom water during ice cover at all three bay stations was about 1 C. Although the main lake station was not visited in the winter, a deepwater temperature of 3–4 C probably occurred (Anderson et al. 1961).

Temperature characteristics of the Big Bay station should be representative of the inner bay and those parts of the middle bay where thermal stratification is not persistent and where depths are similar. Intermittent insulation of deeper waters occurred at Glenora and nearly complete insulation was observed at Conway and at the Lake Ontario station.

Concentrations of dissolved oxygen over the bottom were near air saturation at all stations in the spring. The concentration progressively declined as the water increased in temperature through the summer (Fig. 3), but the degree of saturation also generally decreased. However, no observations of oxygen concentration below 90% saturation were made at Big Bay in 1968.

The minimum observed concentration (mg liter^{-1}) of dissolved oxygen at the four stations in the two years of the study were:

	1967	1968
Big Bay	5.1 (June 14)	8.5 (Aug. 2)
Glenora	3.1 (Sept. 19)	5.8 (Aug. 13)
Conway	3.4 (Sept. 19)	7.1 (Aug. 26)
L. Ontario	–	6.3 (Aug. 27)

Diurnal fluctuation in dissolved oxygen concentration occurred in the water over the mud at Big Bay at times of algal blooms. The maximum and minimum concentrations were 8.4 and 5.3 mg liter^{-1} on August 8–9, 1967, and 15.8 and 11.8 mg liter^{-1} on August 29–30, 1968. Although these results from Big Bay probably were representative of much of the inner bay, local areas of bottom

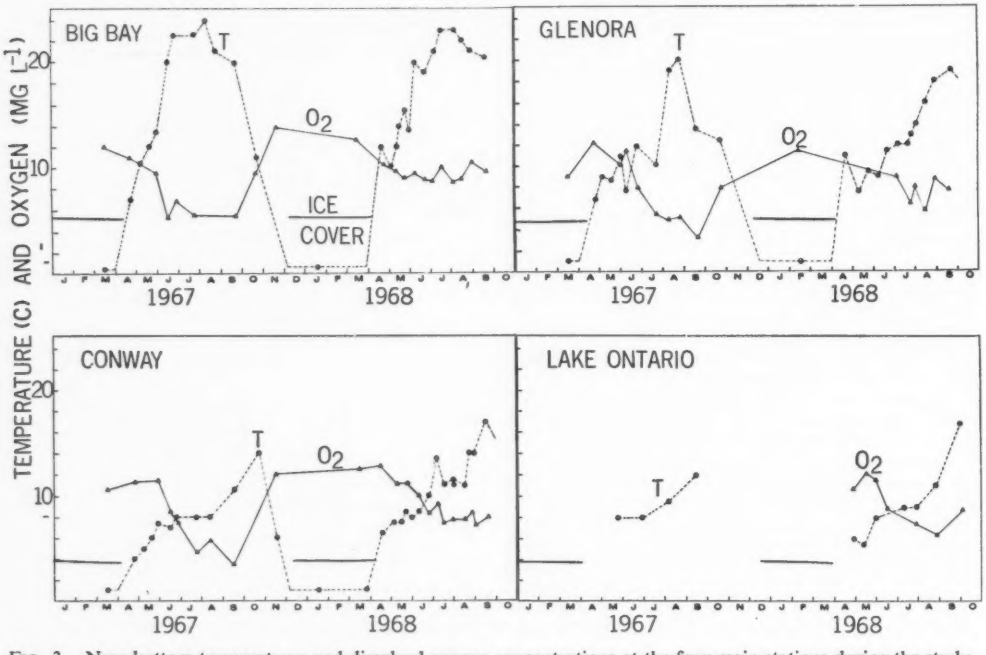

FIG. 3. Near-bottom temperatures and dissolved oxygen concentrations at the four main stations during the study.

water (for example, near Belleville and in Long Reach) may lose most or all of the dissolved oxygen during short calm periods (Dr D. Hurley personal communication).

Respiration of Macroinvertebrates

Respiration rates of the common taxa were measured in relation to ash-free dry weight and temperature (Table 1). All regressions were significant at $P = 0.01$ except for gravid *Pontoporeia affinis*, for which temperature was constant (1 C) and weight did not vary greatly. Although weight occurs on both sides of these equations and validity of the analysis may be questioned on account of this, the alternate form (where R is in μg day^{-1} per animal and validity is ensured) provided similarly satisfactory results.

Insufficient data were available to define statistically the difference in respiration rate between gravid and juvenile stages of both *Asellus racovitzai* and *P. affinis*, but the separation of these physiological stages was deemed advisable. The respiration rates of the two species were remarkably similar with respect to both temperature and individual weight.

Statistically different respiration rates of the three species of oligochaetes could not be combined and used for all species. Pooling of data on sphaeriids was statistically permissible, although the respiration of *Pisidium compressum* was apparently atypical of the group in relation to both temperature and animal weight, probably because of fewer data than for other species. Data from several other groups of unidentified young sphaeriids and representatives of three larger forms (*P. idahoense*, *P. variabile*, and *P. lilljeborgi*) were added to the pooled data to expand the regression with respect to individual weight.

Chironomids were divisible into two general groups, predators and detritivores, following examination of the gut content of thousands of mounted specimens. The former had higher respiration rates than the latter. The main predators examined were *Procladius* spp. and *Coelotanypus concinnus* and the main detritivores were *Chironomus plumosus* and *C. attenuatus* gr. Respiration rates of a few groups of a number of other species, including the detritivores *Chironomus atritibia*, *Crytochironomus* sp., and *Tanytarsus* spp., were examined and appeared to be referable to the detritivore group.

The variation in Q_{10} among the species examined was considerable (Table 2). The coefficient of temperature (a_1 in Table 1) is $\frac{1}{10}$ of the antilogarithm of the Q_{10}, where the latter is the proportionate increase in respiration rate per 10 degree C increase in temperature. There was no clear pattern in Q_{10} with respect to taxonomic groups. Two of the three oligochaetes had low Q_{10} values. Three of four sphaeriids had high values. Crustaceans and chironomids had Q_{10} values between 3.0 and 4.0, all near the average of 3.5 for all species examined.

The coefficient of weight (a_2) was converted to the alternative form (where R is oxygen consumption of the individual at a given weight, rather than oxygen consumption per unit weight) by adding 1.0 to give the T-line, at a given temperature, of Paloheimo and Dickie (1965). This derived exponent of weight showed considerable variability (Table 2),

TABLE 1. Predicting equations for determination of respiration rates of selected macroinvertebrates (μg mg^{-1} day^{-1}) from data on temperature (T) and mean animal ash-free weight (W) in mg. Number of observations is shown (N) and calculated value of F (variance attributable to regression/error variance). G♀ indicates gravid animals.

Species	Equation	N	F
Tubifex tubifex	logR = 2.6126+0.0455T–0.2654 logW	31	35.348**[a]
Limnodrilus hoffmeisteri	logR = 1.8926+0.0512T–0.2428 logW	17	13.063**
Peloscolex ferox	logR = 0.4637+0.1062T–0.6831 logW	10	12.011**
Asellus racovitzai	logR = 1.5901+0.1126T–0.2677 logW	34	159.184**
Asellus racovitzai G♀	logR = 1.5834+0.1209T–0.3295 logW	7	74.969**
Pontoporeia affinis	logR = 1.4782+0.1376T–0.3433 logW	37	108.422**
Pontoporeia affinis G♀	logR = 3.2600 – 0.5640 logW(at1 C)	6	0.821
Chironomus s. s.	logR = 1.6202+0.1146T–0.1861 logW	61	226.621**
Procladius spp.	logR = 2.0240+0.1149T–0.3400 logW	12	56.304**
Pisidium casertanum	logR =–0.3126+0.1926T–0.2684 logW	17	59.717**
P. henslowanum	logR = 0.4176+0.1541T–0.1464 logW	20	68.206**
P. compressum	logR =–0.0651+0.1082T–0.7555 logW	9	13.397**
P. ventricosum	logR =–0.2050+0.1761T–0.3219 logW	7	51.405**

[a] **Highly significant ($P < .01$).

TABLE 2. Mean values of Q_{10} and weight exponents (see Methods) calculated from regression coefficients of temperature and weight. Confidence limits (in parentheses) are ± twice the standard error of correlation coefficient calculated in multiple regression analysis.

Species	Q_{10}	Weight exponent
Tubifex tubifex	1.58(1.40–1.78)	.73(.61–.85)
Limnodrilus hoffmeisteri	1.67(1.34–2.08)	.76(.56–.96)
Peloscolex ferox	2.89(2.25–3.71)	.32(.00–.98)
Asellus racovitzai	3.08(2.65–3.58)	.73(.61–.85)
Asellus racovitzai G♀	3.35(2.62–4.28)	–
Pontoporeai affinis	3.96(3.07–5.09)	.66(.56–.76)
Chironomus s. s.	3.15(2.81–3.52)	.81(.75–.87)
Procladius spp.	3.16(2.45–4.06)	.73(.30–1.00)
Pisidium casertanum	6.86(4.82–9.76)	.73(.31–1.00)
P. henslowanum	4.67(3.57–6.10)	.85(.61–1.00)
P. compressum	2.95(1.62–5.36)	.25(.00–.85)
P. ventricosum	5.80(4.88–6.94)	.68(.40–.96)

but it is probably more consistent among species than Q_{10}. The more precise estimates were between 0.66 and 0.81, and, when the values for *P. ferox* and *P. compressum* were omitted, the mean obtained was 0.74.

The respiration rates of the main species were in general agreement with those in the literature (Table 3) for these and related species.

Standing Stocks

The standing stocks of chironomids were largest at the Glenora and Big Bay stations and declined progressively lakeward (Table 4). Oligochaetes and sphaeriids were least abundant at Big Bay and most abundant at Glenora and declined lakeward. Isopods were rare in the inner bay, appeared to reach peak abundance at Glenora, declined along the gradient into Lake Ontario, and were absent in the deeper (>100 m) sediments of Lake Ontario. Amphipods increased in abundance lakeward beginning at Glenora, and appeared to attain maximum standing stocks in the main deep basin of Prince Edward Bay. They declined at greater depths in Lake Ontario. The total standing stocks were smallest at Big Bay and in the deep basin of Lake Ontario.

The trend toward larger standing stocks from the inner bay into Lake Ontario followed by a decrease in deeper lake sediments also was apparent in data from extensive sampling along 44 transects throughout the study area (Fig. 4). Standing stocks of macroinvertebrates in May–June 1966 averaged 0.7 g m^{-2} in the inner bay, 0.9 g m^{-2} in the middle bay, and 2.2 g m^{-2} in the outer bay. Lake standing stocks that were sampled later in the year, in August of 1967 and 1968, averaged 3.2 g m^{-2}. (The lake data are not directly comparable with data from the outer bay because juveniles of the most abundant species, *Pontoporeia affinis*, were collected in the lake in August but they were missed in the May sampling in the bay.) Biomass in several FWPCA samples from greater than 100 m was 1.8 g m^{-2} indicating that summer stocks were lower in the deep than in the inshore sediments of the lake.

Temporal variation in standing stocks in the main groups of species at the four stations was generally lacking in pattern. The fluctuations were partly the result of sampling variation. Generally, the lower the mean number of animals, the poorer the precision that was obtained. The standard error averaged 19% of the total number of individuals of all species for all stations and sampling times. Therefore, confidence limits ($P < .05$) about total population estimates averaged ±38%. Low and high standing stocks of all animals at each station averaged 60 and 170% of the mean. Therefore, an estimate of standing stock made on a single visit may be only one-half or almost double the annual mean. Furthermore, our data showed no predictable time of year when the standing stocks are close to the annual mean.

The variation in core biomass (removed with fine screen) was too great to obtain sufficiently reliable estimates for comparison with the biomass taken in corresponding dredge samples (removed with a 0.55-mm screen) to determine the efficiency of collecting by regular screening. A second approach, used on chironomids and *P. affinis*, consisted of comparing the length-frequency distribution of animals in a set of cores with the distribution in corresponding regular Ekman samples. Calculated

total biomass of chironomids in cores was 94% of biomass in regular samples. The lowest efficiency of collecting *P. affinis* occurred in April and May at Conway, when 70% of the estimated total biomass was obtained. Similar underestimation of biomass of *Asellus racovitzai* during the summer period of reproduction would be expected.

Instantaneous Growth Rates

The macroinvertebrates examined were divisible into two categories, those in which instantaneous growth rates were related to temperature, as occurred in oligochaetes, sphaeriids, and chironomids and those in which the pattern in growth was best

TABLE 3. Published respiration rates of species, identical with and related to Quinte species, derived from various sources. Published rates are referable usually to live weight; therefore, the ash-free weights are approximate. Rates calculated with estimating equations developed in the present study may be compared, with some caution, with the published rates.

Species	Reference	Estimated ash-free dry wt (*mg*)	Temp (*C*)	Respiration rate ($\mu g\ mg^{-1}\ day^{-1}$)	
				Derived	Calculated
Chironomus anthracinus	Berg et al. 1965, 1962	1.34	8	4	12
		1.28	16	24	30
		0.80	13	73	23
		0.80	13	39	23
Chironomus riparius	Edwards 1958	0.45	10	42	18
		0.90	10	36	16
		1.80	10	29	14
		0.45	20	121	58
		0.90	20	103	51
		1.80	20	77	45
Procladius sp.	Berg et al. 1962	0.35	15	19[a]	61
Chironomus plumosus	Walshe-Maetz 1953	1.00	20	188	80
Tubifex tubifex	Fowler and Goodnight 1965;	0.19	25	131	66
	Berg et al. 1962; Palmer	0.60	11	5[a]	26
	1968 ·	0.04	20	188	80
Pisidium casertanum	Berg et al. 1965, 1962	0.19	8	11	5
		0.26	16	5	23
		0.20	11	3[a]	9
Asellus aquaticus	Edwards and Learner 1960	0.10	20	96	86
		0.50	20	53	57
		1.00	20	48	47
		2.00	20	41	39
		0.50	10	36	18
		1.00	10	29	15
		2.00	10	25	13

[a]Oxygen concentration low (5% air saturation).

TABLE 4. Average standing stocks (g ash-free weight m^{-2}) of the main groups of macroinvertebrates taken at the four main stations of the Quinte study area and in FWPCA samples during 1967–68.

Group	Station				
	Big Bay	Glenora	Conway	Lake Ontario	FWPCA
Chironomids	0.91	2.32	0.24	.09	0.01
Oligochaetes	0.12	1.61	0.36	0.32	0.31
Sphaeriids	Negligible	0.34	0.17	0.17	0.02
Isopods	–	1.16	0.36	0.18	–
Amphipods	–	0.05	3.18	4.24	1.50
Total	1.03	5.48	4.31	5.00	1.84

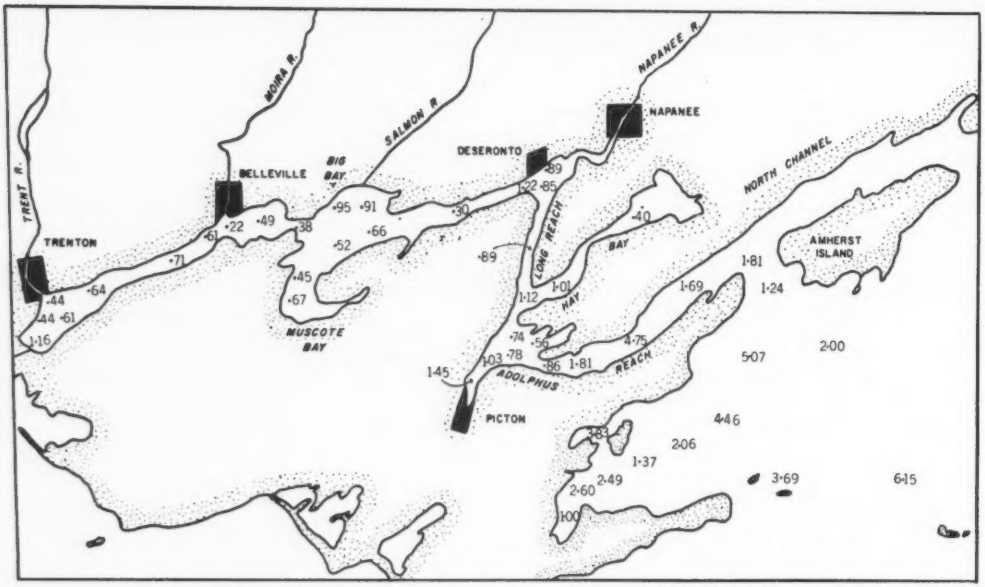

FIG. 4. Standing stocks (g ash-free weight m^{-2}) estimated from extensive sampling at 44 stations in the Bay of Quinte (collected in May and June 1966) and Prince Edward Bay and Lake Ontario (collected in August 1967). Usually six Ekman grab samples were taken on a single visit to each station.

described in relation to the life cycle of the animals, as in *Asellus* and *Pontoporeia*.

Instantaneous growth rates (G) of Big Bay and Glenora oligochaetes were related to temperature (Fig. 5). Q_{10} values were 1.7 for Big Bay and 4.5 for Glenora oligochaetes over the range 1–20 C. Constants of G were calculated for Conway and Lake Ontario oligochaetes. Data were pooled for the three stations where sphaeriids were present to obtain a single equation relating G with temperature (Fig. 6). The Q_{10} over the range 1–20 C was 2.0. Detritivore and carnivore chironomids had lower and higher values of G, respectively, but differences among stations were not apparent in the correlation of G with temperature (Fig. 7). Q_{10} values were 6.1 for detritivores over 1–20 C and 5.4 for carnivores over 6–20 C. G of *Chironomus* species was assigned a value of zero at and below 5 C (as opposed to negative values given by the predicting equation) because weight losses determined from field data were negligible at <5 C.

For *P. affinis* at the Conway and Lake Ontario stations, the values of G were highest in the spring of the first year of life and declined markedly by the fall (Fig. 8). During the 2nd year the values increased somewhat until spring and by fall were lower than a year earlier. No growth occurred in gravid *P. affinis* in January through March. Although the weight of nongravid animals in early fall was less than that of the gravid animals in winter, the absolute caloric contents of the two were approximately the same. The former contained proportionately more oil than the latter. Presumably production of young was based on the equivalent of stored energy, and the equivalent of metabolic requirements was assimilated through the reproductive period. Therefore, production of gravid individuals was taken as zero and their metabolic requirement was included in assimilation. The decline in weight and caloric content after reproduction exceeded the energy lost in young. Moreover, the numerous dead and emaciated animals in April samples indicated that feeding ceased and death occurred following reproduction. Therefore, assimilation of postreproductive animals was assumed to be zero.

Instantaneous growth rates of *Asellus* were related to life-history stages. G was high in young animals in summer (Fig. 9) and declined through fall and early winter. G increased in spring just before the reproductive season. Calculation of G during the reproductive season was hindered by differential mortality because the larger males and females died. The largest females became gravid in April, and young were found in core samples for the first time

about 120 days thereafter. A similar gestation period occurred in the small females in the July cohort. During 120 days, females increased in weight from 0.70 to 2.45 mg, giving G a value of 0.01. Males, examined in the same manner, had G of 0.02. Similar values were obtained when groups of males were held in native sediments in the laboratory. The few postreproductive and emaciated females collected were assumed to have ceased growing and feeding, that is, assimilation probably was zero.

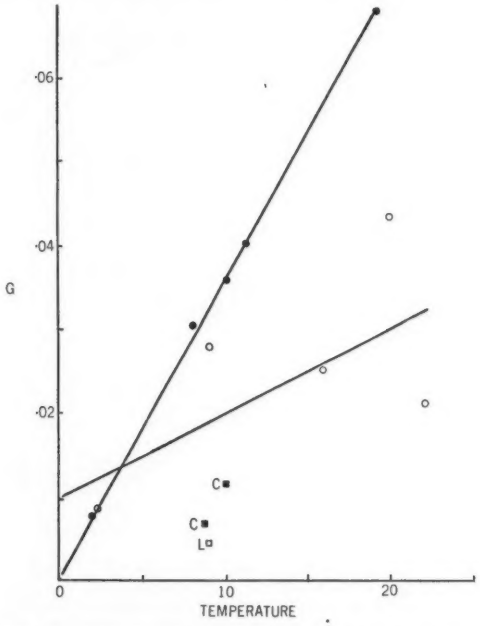

FIG. 5. Instantaneous growth rates (G) of oligochaetes in relation to temperature. Data were obtained from laboratory "cohorts." Oligochaetes and native muds were from Big Bay (open circles), Glenora (solid circles), Conway (C), and the Lake Ontario station (L). Lines represent the equations calculated with least-squares method (r = 0.99 for Big Bay and 0.66 for Glenora).

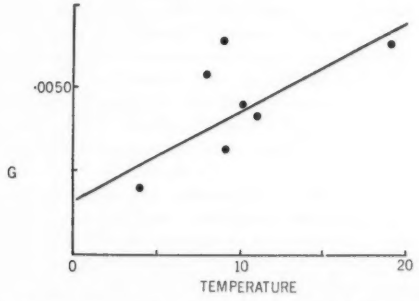

FIG. 6. Instantaneous growth rates (G) of sphaeriids in relation to temperature. Data were obtained from laboratory "cohorts," from Glenora and Conway. Line represents the equation calculated with least-squares method (r = 0.60).

Production and Assimilation

Production and respiration of macroinvertebrates at each of the four stations (Fig. 10) showed the same pattern throughout the year. Pronounced summer maxima occurred. The peaks were greater in 1968 than in 1967 at each station. Winter minima divided the study period into two growing seasons. The magnitude of production in the 1968 growing season is evident without fall data.

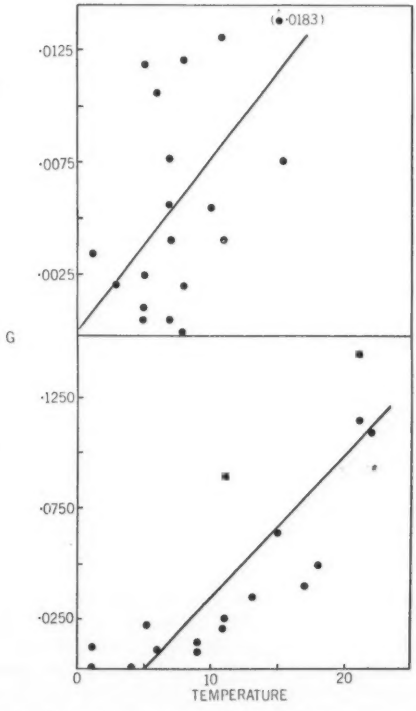

FIG. 7. Instantaneous growth rates (G) of chironomids in relation to temperature. Data were obtained from cohorts recognized in field samples taken at the three Bay of Quinte stations. *Upper panel* — carnivores; *lower panel* — detritivores. Lines represent the equations calculated with least-squares method (r = 0.53 for carnivores and 0.83 for detritivores)

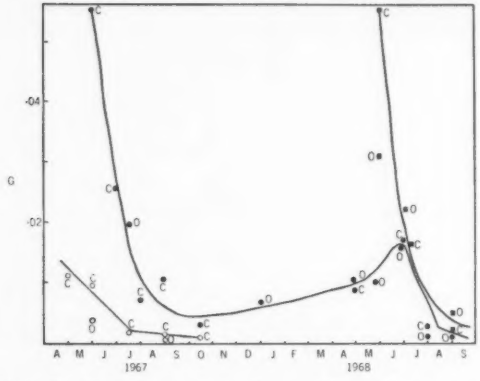

FIG. 8. Instantaneous growth rates (G) of *Pontoporeia affinis*. Open circles, the 1966 year-class (age 1) in 1967; solid circles, the 1967 year-class; squares, the 1968 year-class. Data are from field cohorts at Conway (C) and the Lake Ontario station (O).

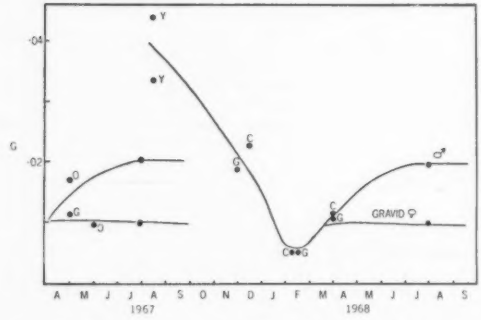

FIG. 9. Instantaneous growth rates (G) of *Asellus racovitzai*. Circles at left, the 1966 year-class (age 1) in 1967, males (*upper curve*) and females (*lower curve*); circles at right, the 1967 year-class, including newborn grown in native sediments in the laboratory (Y). Data are from field cohorts at Glenora (G) and Conway (C) and estimated growth rates of breeding males and females.

Production of macroinvertebrates was greatest at Glenora, followed in order by the Big Bay, Conway, and Lake Ontario stations. The average assimilation and production at the four stations (kcal m^{-2} day^{-1}) over the 18-month study period were:

Station	Assimilation	Production
Big Bay	0.35	0.26
Glenora	1.18	0.80
Conway	0.46	0.19
L. Ontario	0.42	0.16

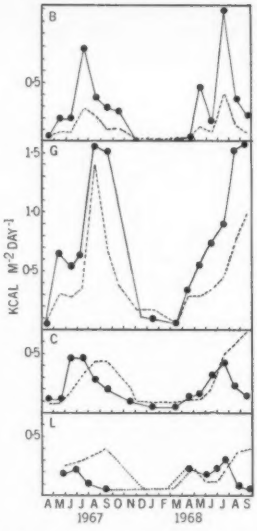

FIG. 10. Production (circles) and respiration (dashed line) of macroinvertebrates at the four main stations through the period of study.

Estimates of production and assimilation by macroinvertebrates in the deep profundal of Lake Ontario, although based on minimal data (FWPCA samples), were 0.037 and 0.087 kcal m^{-2} day^{-1}, respectively. Most of this production was by *P. affinis* (0.025 kcal m^{-2} day^{-1}) and oligochaetes (0.011 kcal m^{-2} day^{-1}).

The species that accounted for most of the production at each of the stations were, in descending order: Big Bay — *Chironomus plumosus*, *Chironomus* sp. A, *C. attenuatus*, *Limnodrilus hoffmeisteri*, *Procladius* spp., and *Coelotanypus concinnus*; Glenora — *Tubifex tubifex*, *Chironomus anthracinus*, *C. attenuatus*, *Asellus racovitzai*, and *Limnodrilus hoffmeisteri*; Conway — *Pontoporeia affinis*, *Limnodrilus hoffmeisteri*, and *Asellus racovitzai*; Lake Ontario main station — *P. affinis*, *L. hoffmeisteri*; deep Lake Ontario — *P. affinis* and *Stylodrilus heringianus*.

Annual rates of production and assimilation of common macroinvertebrate taxa were calculated for the two overlapping periods April 1967–March 1968 and October 1967–September 1968 (Table 5). Though rates for the two periods are not independent, they were determined largely by their respective periods of greatest production, the separate May to September periods (Fig. 10).

Crustaceans were equally productive at the Glenora, Conway, and Lake Ontario stations.

TABLE 5. Average biomass, or standing stocks ($\bar{B}$), total production (P), respiration (R), and assimilation (A), and calculated turnover ratios (P ÷ $\bar{B}$) and growth efficiencies (P ÷ A) during two 1-year periods at the four main stations.

Station and group	First period						Second period					
	$\bar{B}$	P	R	A	TR	E	$\bar{B}$	P	R	A	TR	E
Big Bay												
Chironomids	4.43	67.6	31.4	99.0	15.3	.68	5.19	72.6	41.4	114.0	14.0	.59
Oligochaetes	1.02	6.7	3.0	9.7	6.5	.68	0.91	5.2	2.3	7.5	5.7	.69
All groups	5.45	74.3	34.4	108.7	13.6	.68	6.10	77.7	43.6	121.5	12.7	.59
Glenora												
Crustaceans	6.81	43.6	32.9	76.5	6.4	.57	3.96	23.5	13.6	37.1	5.9	.63
Chironomids	13.93	92.7	41.0	133.7	6.7	.69	16.54	162.7	55.4	218.1	9.8	.74
Oligochaetes	7.62	94.0	40.7	134.6	12.3	.70	7.83	86.0	44.4	130.4	11.0	.66
Sphaeriids	1.57	2.4	20.9	23.3	1.5	.10	1.76	2.7	22.0	24.7	1.5	.11
All groups	29.93	232.7	135.5	368.2	7.8	.64	30.09	274.9	135.4	410.2	9.2	.67
Conway												
Crustaceans	22.17	56.4	63.9	120.3	2.5	.47	21.59	45.7	80.1	125.8	2.1	.36
Chironomids	1.27	4.9	3.4	8.2	3.8	.59	1.18	7.7	3.9	11.6	6.5	.66
Oligochaetes	1.17	3.4	3.8	7.2	2.9	.48	2.00	5.8	6.6	12.4	2.9	.47
Sphaeriids	0.94	1.1	4.7	5.8	1.2	.19	0.90	1.2	7.7	8.9	1.3	.14
All groups	25.55	65.8	75.8	141.6	2.6	..47	25.67	60.4	98.3	158.7	2.4	.38
Lake Ontario												
Crustaceans	36.46	46.2	101.1	147.3	1.3	.31	23.39	60.3	74.6	134.9	2.6	.45
Chironomids	0.47	1.4	2.8	4.2	2.9	.33	0.52	1.7	2.6	4.2	3.2	.40
Oligochaetes	1.02	1.8	3.1	4.9	1.8	.38	2.16	3.9	5.3	9.2	1.8	.42
Sphaeriids	0.74	1.2	7.6	8.8	1.4	.12	1.11	1.6	7.3	8.9	1.4	.18
All groups	38.69	50.6	114.6	165.2	1.3	.31	27.18	67.5	89.8	157.3	2.5	.43

However, *A. racovitzai* was the only crustacean of significance at Glenora, and *P. affinis* was most important at the Lake Ontario station. Both species were important at Conway. *Asellus* was absent in deepwater FWPCA samples. The turnover ratio of crustaceans declined from about 6 at Glenora to 2 at Conway and the Lake Ontario station and to about 1 in the deep waters of Lake Ontario. Chironomids accounted for about 90% of macroinvertebrate production at the Big Bay station and had annual turnover ratios of about 15 times. The importance of chironomids in production and their turnover ratios declined lakeward (Table 5).

Oligochaetes contributed most to production, about 35% of the total, and had their highest turnover ratios, 12 times per year, at Glenora. Their turnover ratio was 2.9 at Conway and 1.8 at the Lake Ontario station.

Sphaeriids had low turnover ratios, between 1 and 1.5, at all stations where they were present. The contribution by sphaeriids to total production was relatively low at all the main stations, although extensive sampling indicated greater standing stocks, particularly of filter-feeding *Sphaerium* spp., in sublittoral sandy sediments than at the main stations.

Turnover ratios of all macroinvertebrates at each station declined progressively from 13 in Big Bay to about 1 in the deep waters of Lake Ontario.

Growth Efficiencies

Patterns in growth efficiency in *Asellus racovitzai* and *Pontoporeia affinis* (Table 5) were similar in part. Young of both species had high growth efficiencies (0.7–0.8), which declined with increasing weight. However, during reproduction, growth efficiency of *Asellus* was approximately 0.3, whereas reproducing *Pontoporeia* did not grow. Teal (1957) calculated a growth efficiency of 0.20 for *A. militaris*; no published efficiencies on *Pontoporeia* were found. Collectively the two species had growth efficiencies of approximately 0.6 at Glenora and 0.4 at Conway and the Lake Ontario station.

Predatory chironomids had lower growth efficiency than the detritivore chironomids: 0.2–0.3 and 0.6–0.7, respectively, at temperatures above 5 C. Low values may be expected in predators (Lindeman 1941; Welch 1968; Kozlovsky 1968). Growth efficiency of chironomids collectively over

the entire study period, including one winter, was 0.5 with little variation among stations.

The growth efficiency of oligochaetes in the study area averaged about 0.50 but was greatest at 0.68 at Big Bay and declined progressively lakeward to 0.35 in Lake Ontario. Ivlev (1939) demonstrated a growth efficiency of 0.62 in *Tubifex tubifex* at 20 C and Teal (1957) gave 0.26 at 10 C for springpond *Limnodrilus hoffmeisteri*. The values at higher temperatures (0.70–0.76) in the present study seem rather high and could be in error, but growth efficiencies at intermediate temperatures were generally in agreement with Ivlev's estimates. There is no a priori reason to invalidate values of 0.6–0.7 because the animals in question exist literally in a food bath delivered by gravity flow. In fact, net growth efficiencies of this magnitude are not unusual, although they are accompanied generally by low assimilation efficiencies, which would be expected in mud-dwelling detritivores (table 1 and fig. 1 in Welch 1968).

Sphaeriids had low growth efficiencies that averaged about 0.2. Teal (1957) found a net growth efficiency of 0.47 in *Pisidium virginicum* (=*P. dubium*) and *Sphaerium partumeium* in his 10 C spring, but no other published growth efficiencies in sphaeriids were found.

Growth efficiencies of the macroinvertebrates ("All groups", Table 5) declined from Big Bay lakeward. This apparently resulted from declines in efficiencies in each group present as well as from the effect of the increased proportion of crustaceans comprising the association at lakeward stations.

Discussion

Production and assimilation by macroinvertebrates apparently were greatest in Adolphus Reach and lower in the inner bay, contrary to what might be expected from decreasingly eutrophic conditions (Johnson and Owen 1971) between the inner bay and Lake Ontario. In fact, assimilation by macroinvertebrates was greater at the main station in Lake Ontario than at the Big Bay station in the inner bay. Production at the latter was only about one-third greater than at the Lake Ontario station, and this difference appeared to be the result of respective higher and lower growth efficiencies of *Chironomus*- and *Pontoporeia*-dominated associations.

This relation between production and state of eutrophication is not explained by import of detritus to bottom sediments, which declined between the inner bay and lake (Johnson and Brinkhurst 1971b), unless qualitative differences in detritus are examined. Macroinvertebrates utilized a small proportion (2%) of the energy in detritus in the inner bay and a much larger proportion (about 30%) in the outer bay and lake. Sedimentation of detritus has not been measured in the main basin of Lake Ontario, but the decline in macroinvertebrate production from inshore to offshore areas observed in the present study probably was related to decreased sedimentation and increased settling time offshore.

Turnover ratios of macroinvertebrates were closely related to annual mean temperature (Fig. 11), although turnover ratios of taxonomic groups varied. Temperature appeared to have a fundamental controlling influence on turnover ratio of the whole macroinvertebrate community as it affected the respiration and growth of individuals within the community. That is, biomass B appeared to be adjusted to the utilizable import, consistent with a turnover ratio set by temperature. The relation, taken from Fig. 11, is given approximately by

$$TR = \frac{T^2}{10},$$

where TR is annual turnover rate and T is mean temperature (C). Production, therefore, is given by

$$P = B\,\frac{T^2}{10}.$$

Published data on turnover rates add support to the present findings and agree generally with the relation found between turnover ratio and temperature. In Ontario trout lakes, chironomid standing stocks were replaced 8–9 times each year in the shallows and 2–3 times in the deeper colder waters (Miller 1941). In Grosser Plöner See, the annual turnover of benthic macroinvertebrates was 3–4 times (Lundbeck 1926). In profundal Lake Beloie,

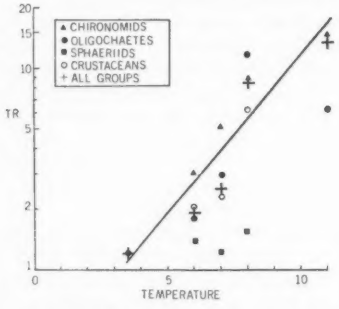

FIG. 11. Relation between annual mean temperature (C) and the turnover ratio (TR) of the macroinvertebrate community and of broad taxonomic groups.

Borutsky (1939) found that the standing stocks turned over approximately twice annually. Higher values have been recorded at the warmer temperatures of ponds; for example, in two Michigan ponds, fish removed 8–27 times the average standing stocks (Hayne and Ball 1956), and in a fertilized Georgia pond, Welch (MS 1967) demonstrated that the standing stocks turned over every 6–7 days.

Standing stock sometimes has been used as an index of productivity, although the danger generally is recognized. Standing stock and production were not closely related in the present study. However, our data indicate that standing stock values, together with data on mean temperature, may be useful in making inferences about macroinvertebrate production in the Great Lakes.

We have been tempted to speculate on the usefulness of a physiological basis for measuring production. Production and assimilation may be calculated from data on respiration and growth efficiency. Difficulties were encountered in determining production in the present study, particularly in following cohorts over extended periods, which necessitated removing animals to the laboratory for measurement of growth in the case of oligochaetes and sphaeriids.

Respiration rates of each important species would have to be measured at required temperature–body weight combinations or by development of appropriate families of T-lines (Paloheimo and Dickie 1965). Although similarities in respiration rates were greater than expected in a taxonomically diverse group, there is probably little scope for further generalization, except perhaps for some species groups, for example, sphaeriids. The constant a_0 gave only a rough indication of the general level of respiration. For example, the oligochaete *T. tubifex* and the chironomids *Procladius* spp. had the greatest values of a_0. The former, because of its low Q_{10} (Table 2), did not have a relatively high respiration rate. Although the respiration rate of *T. tubifex* was greater than that of all other species examined at the lower temperatures, it was, in fact, lower than average at the higher temperatures. *Procladius* spp., however, had an average Q_{10} of about 3 and, together with the high value of a_0, realized a consistently high respiration rate. This example indicates the marginal utility of the a_0 value alone. The average weight exponent in this study (0.74) was similar to published values, for example, 0.71 in *Chironomus riparius* (Edwards 1958), 0.70 in *Asellus aquaticus* (Edwards and Learner 1960), and a mean of 0.70 in 12 snails (Berg and Ockelmann 1959). Therefore, a generalized weight exponent could be of value, but wide and unsystematic variation of Q_{10} is probable. Berthet (1963) found a variation from 2.6 to 5.6 in the Q_{10} of 16 soil mites and Knight and Gaufin (1966) gave 0.6 and 3.2 as the lowest and highest Q_{10} in several species of stoneflies. Such variation places some reservations on the value of any current general scheme for collectively estimating the respiration of groups of species, even if the parameter a_0 could be used to scale the intensity of metabolism set by nutritional and taxonomic status. The interaction among species remains a problem; for example, one of the authors (ROB) has found significantly lower respiration in mixed oligochaete cultures than in single-species cultures at the same density. Also, we must determine the relation between respiration rates measured under various conditions in the laboratory (e.g., Erikson 1963 who used sediments of different texture) and respiration rates under natural conditions.

Growth efficiencies for each important species and life-history stage may be obtained from field or laboratory data. Probably there are no simple responses of growth efficiency to variables such as temperature, animal weight, and food supply, which can be applied to a variety of species. The temporal changes in growth efficiency in *Asellus racovitzai* and *Pontoporeia affinis* indicate the sort of complexity that may occur. Yet, the average growth efficiencies of these two species and a variety of species of oligochaetes and nonpredatory chironomids were between 0.3 and 0.6, with many values near 0.5. Mean annual growth efficiencies of macroinvertebrates at each station varied from 0.35 to 0.68, and the lower values within this range were explained in part by the high proportion of crustaceans. Such information, together with data on respiration, would allow approximate measurement of macroinvertebrate assimilation and production.

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Appendix 1

Relation among Length, Weight, and Calories in Macroinvertebrates from the Bay of Quinte and Lake Ontario

Most of the data are from selected groups of animals of even size that had been used in respiration studies, and, therefore, had not been feeding during the previous 4–5 hr. The ash-free weight as a percentage of total dry weight varied between 14% in the heavy-shelled sphaeriid *Pisidium variabile* to about 90% in most chironomids. A mean value of about 20% was characteristic of most sphaeriids, although small sphaeriids generally contained a greater percentage of organic matter than larger individuals of the same species. The average percentages of ash-free weight for several species were as follows: *P. henslowanum* 21%, *P. casertanum* 19, *P. compressum* 22, *P. ventricosum* 19, *P. idahoense* 18, *P. variabile* 14.

The average percentage of ash-free weight of chironomids and oligochaetes was 90%. Relative ash-free weight increased with total weight in *Pontoporeia affinis* and decreased in *Asellus racovitzai*. About 74 and 64% ash-free weight were found in immature and mature *Asellus* respectively; 80 and 87% ash-free weight were observed in immature and mature *Pontoporeia*.

Regressions of ash-free weight on body length (App. Table 1) were obtained for all groups (except oligochaetes) whose population respiration and net production were calculated.

The general conformation of the main species of chironomids permitted the use of a single estimating equation. Greater variation was apparent among sphaeriids. For example, the robust *P. ventricosum* is almost twice as heavy as *P. henslowanum* at a given length. Most of the common sphaeriids are of similar proportions, such as *P. henslowanum*, *P. casertanum*, and *P. compressum*. However, a general length–weight relation was used, with appreciation of bias introduced into the weights of the robust forms such as *P. ventricosum* and *P. variabile*. Because of the lesser abundance of the latter group, no large error was introduced into biomass, respiration, and production estimates of sphaeriids as a group. The gravid and postreproductive females of both *P. affinis* and *A. racovitzai* were heavier and lighter respectively than juveniles and males at a given length. Therefore, separate estimating equations were required for these life stages (App. Table 1).

Calculation of biomass from length-frequency distributions of preserved animals and length–weight functions based on fresh, 60 C-dried animals, as carried out in this study, is preferable to direct weighing of preserved animals. Preservation apparently extracts organic matter. Actual ash-free weights of oligochaetes in four trials were 1.36 times ash-free weights of preserved animals, 1.52 times in *Pontoporeia*, 1.31 times in *Asellus*, and 1.71 times in *Chironomus*. No difference was noted in the case of sphaeriids. The preserved animals were held in 10% formalin for 3 weeks and subsequently in 70% ethyl alcohol for several weeks. This indicates that published data on standing stocks obtained from weights of preserved animals may be biased.

The use of specific caloric values for macroinvertebrates was required in view of the variation observed in the present study (Table 2). Although the mean value of chironomids (5.8 cal mg^{-1}) and oligochaetes and sphaeriids (5.5 cal mg^{-1}) were close to the general mean, the caloric content of *P. affinis* was relatively high at 6.6 cal mg^{-1} and that of *A. militaris* was lower than average at 5.1 cal mg^{-1} ash-free weight. The higher energy content of *P. affinis* was due to the high oil content, which was visually obvious in the form of droplets. These data agree reasonably well with what few values on the same or similar species are available in the literature (summarized by Cummins 1967).

APPENDIX TABLE 1. Length–weight equations for several common macroinvertebrates of the study area. The number of observations (N) and value of F (variance attributable to regression/error variance) are shown for each regression. G and S indicate gravid and spent animals.

Species	Equation	N	F
Asellus racovitzai	logW = −4.366+2.489 logL	18	611.7**[a]
Asellus racovitzai G♀	logW = −2.652+1.709 logL	4	75.8*
Asellus racovitzai S♀	logW = −4.856+2.501 logL	5	12.0*
Pontoporeia affinis	logW = −4.279+2.550 logL	14	257.3**
Pontoporeia affinis S♀	logW = −2.994+1.530 logL	4	25.3*
Gammarus fasciatus	logW = −4.264+2.444 logL	7	253.8**
Procladius spp.	logW = −5.413+2.275 logL	6	135.6**
Coelotanypus concinnus	logW = −5.576+2.207 logL	6	68.0**
Chironomus attenuatus gr.	logW = −4.906+2.032 logL	8	29.0**
Chironomus atritibia	logW = −5.598+2.345 logL	5	16.8**
Chironomus plumosus	logW = −11.364+4.314 logL	9	90.4**
Chironomus digitatus	logW = −8.736+3.779 logL	6	39.1**
Pisidium casertanum	logW = −4.015+2.694 logL	14	119.2**
Pisidium henslowanum	logW = −4.439+2.982 logL	15	1549.5**
Pisidium compressum	logW = −5.020+3.752 logL	8	211.1**
Pisidium ventricosum	logW = −3.642+2.719 logL	7	63.7**

[a] *Significant, $P < 0.05$; **highly significant, $P < 0.01$.

APPENDIX TABLE 2. Caloric values of the macroinvertebrates. The source of material, time of year collected, and, wherever noted, age and condition of animals are indicated.

Taxon	Station	Date	Calorie content (*cal mg*$^{-1}$) Dry wt	Ash-free wt
A. racovitzai G ♀	L. Ontario	July 68	4.12	5.65
A. racovitzai G ♀	Glenora	Aug. 68	4.45	4.95
A. racovitzai ♂	"	Aug. 68	3.84	5.91
"	"	Jan. 69	5.24	5.24
"	"	July 68	3.00	4.62
A. racovitzai young	"	Aug. 68	3.84	5.91
A. racovitzai (mean of above)				5.10
P. affinis G ♀	Conway	Mar. 68	4.71	5.94
"	"	"	4.54	5.72
"	"	"	4.90	6.27
P. affinis young	"	Aug. 68	5.99	7.36
P. affinis young	"	"	6.23	7.78
P. affinis juvenile	"	"	6.50	7.47
"	"	"	6.49	5.74
"	"	"	6.16	7.08
"	"	"	5.35	6.15
P. affinis (mean of above)				6.61
C. plumosus	Glenora	Aug. 68	4.70	5.12
"	"	Aug. 68	5.02	5.41
"	"	Jan. 69	5.84	6.47
"	"	Jan. 69	4.34	4.82
C. attenuatus gr.	"	Aug. 68	5.63	6.25
"	"	Aug. 68	5.98	6.64
"	"	Jan. 69	5.00	5.50
Procladius spp.	"	July 68	5.02	5.57
Procladius spp.	"	Jan. 69	6.40	6.48
Chironomids (mean of above)				5.80
Pisidium spp.	Glenora	Jan. 69	1.01	5.05
"	"	"	0.93	4.65
"	"	"	1.36	6.80
P. casertanum	"	Sept. 68	1.21	6.05
P. henslowanum	"	Sept. 68	0.87	4.35
S. striatinum	"	Jan. 69	1.06	5.30
Sphaeriids (mean of above)			1.07	5.45
T. tubifex–P. bavaricus	Glenora	Aug. 68	5.27	5.38
T. tubifex–P. bavaricus	Glenora	Aug. 68	5.36	5.46
P. ferox	L. Ontario	July 68	5.05	5.60
L. hoffmeisteri	Conway	Aug. 68	5.20	5.36
L. hoffmeisteri	Glenora	June 68	5.29	5.45
T. tubifex–P. bavaricus	Glenora	June 68	5.57	5.73
Tubificids (mean of above)			5.29	5.46

Benthic Community Metabolism in Bay of Quinte and Lake Ontario[1]

M. G. JOHNSON

Department of Zoology
University of Guelph, Guelph, Ont.

AND R. O. BRINKHURST

Department of Zoology
University of Toronto, Toronto, Ont.

JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Benthic community metabolism in Bay of Quinte and Lake Ontario. J. Fish. Res. Bd. Canada 28: 1715–1725.

Rates of sedimentation of organic matter to the bottom sediments at four stations (in order from the inner Bay of Quinte to open Lake Ontario) averaged 1.91, 0.42, 0.36, and 0.21 g m^{-2} day^{-1}. Respective respiration rates of sediment cores averaged 0.35, 0.25, 0.22, and 0.15 g O_2 m^{-2} day^{-1}. Approximately 90% of imported energy (IM) was used by the benthic communities at the three lakeward stations and 23% at the inner bay station. Low utilization in the inner bay was substantiated by the high organic matter (28%) in sediments there, in contrast with Lake Ontario sediments (3–4%). Low utilization was attributed to the relatively greater proportion of allochthonous import. Only 2% of imported energy was assimilated by macroinvertebrates in the inner bay, in contrast with about 30% at the lakeward stations.

A concise model of macroinvertebrate production, $P = c(a \cdot b\ IM)$ (where c is the proportion of growth to respired and exported energy and is derived from the average growth efficiency of macroinvertebrates, a is proportion of imported energy used by the total benthic community, and b is proportion of the latter used by macroinvertebrates), was used to deduce the relation between production and import. All of the parameters a, b, and c presumably decrease with increasing import, although c may be low initially at low import and sparse food supply.

JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Benthic community metabolism in Bay of Quinte and Lake Ontario. J. Fish. Res. Bd. Canada 28: 1715–1725.

Le taux de sédimentation de la matière organique à quatre stations (allant de l'intérieur de la baie de Quinte vers le large du lac Ontario) est en moyenne de 1.91, 0.42, 0.36, et 0.21 g m^{-2} $jour^{-1}$. Le taux respiratoire des carottes sédimentaires a une valeur moyenne de 0.35, 0.25, 0.22, et 0.15 g O_2 m^{-2} $jour^{-1}$ respectivement. Les communautés benthiques des trois stations proches du lac utilisent environ 90% de l'énergie importée (IM), alors que celles de la zone intérieure de la baie n'en utilisent que 23%. Cette faible utilisation se réflète dans les sédiments de la zone intérieure qui ont une forte teneur en matière organique (28%) en comparaison de ceux du lac Ontario (3–4%). Nous attribuons ce bas niveau d'utilisation à un apport relativement élevé de matériel allochtone. Les macroinvertébrés de l'intérieur de la baie n'assimilent que 2% de l'énergie importée à l'opposé d'environ 30% pour ceux des stations proches du lac.

Nous avons établi la relation entre la production des macroinvertébrés et l'énergie importée suivant l'équation concise: $P = c(a \cdot b\ IM)$, dans laquelle c représente le rapport croissance–respiration et énergie exportée calculé à partir du rendement moyen de croissance des macroinvertébrés, a représente la proportion de l'énergie importée utilisée par la totalité de la communauté benthique, et b, celle utilisée par les macroinvertébrés. A priori, toutes les valeurs de a, b, et c diminuent à mesure que l'énergie importée augmente, bien qu'au début c peut être faible, si l'énergie importée est minime et la nourriture peu abondante.

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[1]This paper is the third in a series of three on the benthos of the Bay of Quinte and the adjoining part of Lake Ontario.

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ALTHOUGH descriptive studies on benthic macroinvertebrate communities of the St. Lawrence Great Lakes are important, there is a growing interest in their functional aspects, for example, geochemical cycling, production of fish food, and particularly their part in community metabolism. The last aspect has been outlined in general terms and with specific examples by Odum (1968), Golley (1968), Engelmann (1966 and 1968), Darnell (1968), and Slobodkin (1962). The purpose of our study was to integrate measures of import of organic matter (and energy) to the bottom sediments, flow, and use of energy within compartments of the benthic community and export of energy from the benthos. The Bay of Quinte and the adjacent area of Lake Ontario were selected as the study area because of the trophic gradient there, from the eutrophic inner bay to the depth-dependent oligotrophic conditions of Lake Ontario. Limnological conditions in this study area have been described by McCombie (1966 and 1967), Johnson and Owen (1971), and Johnson and Brinkhurst (1971a), and production and assimilation of energy by macroinvertebrates by Johnson and Brinkhurst (1971b).

The research was carried out at four stations (Fig. 1). The Big Bay station represented the macroinvertebrate association of the inner and middle bays. Chironomids, particularly of the genus *Chironomus*, and in terms of energy flow, *C. plumosus*, were dominant. The Glenora station was in a transitional zone between the above association and a second in Adolphus Reach and Prince Edward Bay. The latter association, sampled at Conway, contained many species of sphaeriids, oligochaetes, chironomids, and crustaceans. The association at the Lake Ontario station was much like the cold-stenotherm association found throughout most of the Great Lakes. The amphipod *Pontoporeia affinis* was the dominant macroinvertebrate, accompanied by the common oligochaetes *Stylodrilus heringianus*, *Limnodrilus hoffmeisteri*, and *Tubifex tubifex*, the sphaeriid *Pisidium conventus*, and the chironomid *Heterotrissocladius subpilosus* (Johnson and Brinkhurst 1971a).

Methods

RATE OF SEDIMENTATION

The rate of sedimentation of detritus to the bottom was estimated by catching detritus in 20-cm diam funnel traps (Fig. 2A) suspended 1.5 m above the bottom at the four stations. Sedimentation was measured in 1967 from mid-April almost to the time of ice formation at the bay stations. Sediment collectors were reset in January and removed again in mid-March, then reset as soon as possible after the ice melted in early April

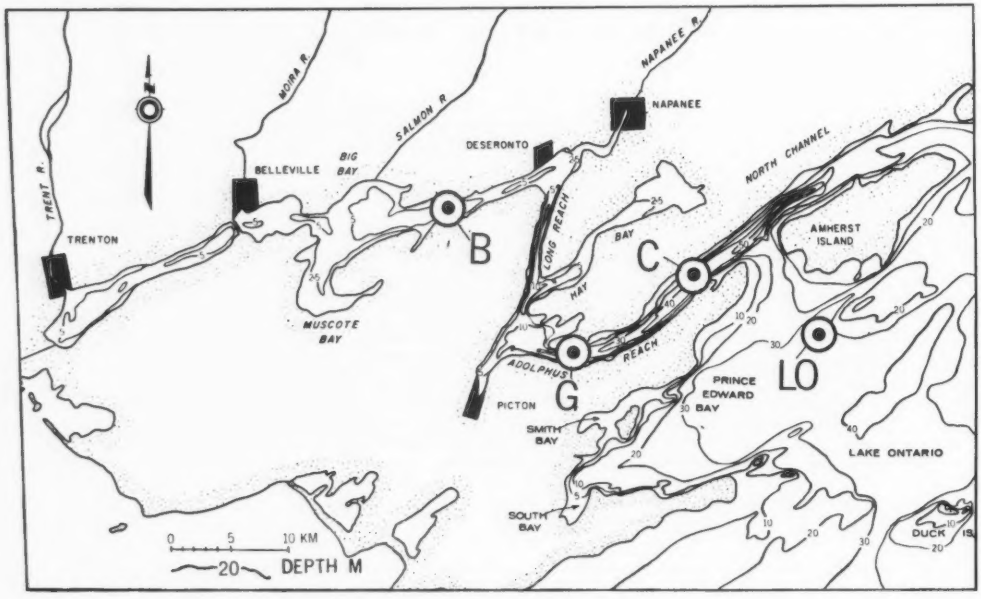

FIG. 1. Location of the study area and the four main stations.

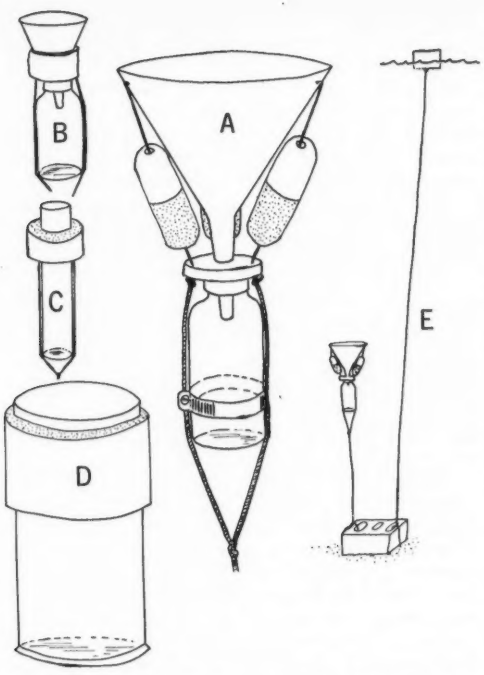

FIG. 2. Sediment-collecting equipment. A, 20-cm diam funnel trap, the one used throughout the study. Gillnet floats provided buoyancy; B, 12-cm diam funnel trap; C, 5-cm cylinder trap; D, 17-cm cylinder trap; E, positioning of sediment traps.

1968. Interpolation of sedimentation rate was required at bay stations for a few periods. Sedimentation was measured at the lake station through 111 days in 1967 and 243 days in 1968. The traps were emptied weekly during the spring and summer and as often as possible at other times. The setting and lifting of these traps did not introduce lake-bottom sediment, as shown by the lack of detritus in traps lifted immediately after they were set out and by the clarity of the supernatant observed in traps as they were retrieved routinely. The contents were preserved with 10% v/v formalin (except frozen subsamples for calorimetry) and, after settling, the volume of each sample was reduced to 100 ml. The loss of solids in the supernatant, which was examined in 17 samples, was 0.3–1.3% of the total.

To estimate the loss of organic matter (and energy) within the traps, because of the delay in collecting the material, the sum of daily collections in a standard trap was compared with a 6-day collection at Glenora in July 1968. Also, the respiration rates of several samples of fresh sediment were measured to estimate the daily loss of energy through microbial metabolism. The sum of *organic* matter in separate collections on 6 consecutive days at Glenora yielded an estimate of the deposition rate of 0.75 g m^{-2} day^{-1}, whereas the cumulative 6-day collection gave a rate of 0.52 g m^{-2} day^{-1}. The deposition rates of *inorganic* material were identical, 1.30 g m^{-2} day^{-1}, from the 6-day collection and the sum of daily collections. The daily loss of energy through microbiological respiration in fresh detritus was about 5% at 9–14 C, as determined by replicated measurements of respiration of six samples from the deeper stations. Therefore, the predicted decline in organic matter accumulating at the rate of 0.75 g m^{-2} day^{-1} by the end of a 6-day period was about 16%. That is, a daily rate of sedimentation of 0.63 g m^{-2} was predicted, which was greater than that obtained by doing the experiment directly by daily collection. (However, the predicted rate was based on the assumption that the sedimentation rate was constant over the 6 days.) The loss of energy in detained sediment was estimated to be 15–25%. Theoretically, a correction was required; therefore, a factor of 1.2 was used to calculate all rates of import of energy. A constant factor should be satisfactory for the purpose of the present study because sampling frequency was related to water temperature, that is, sediment was collected over short intervals when temperatures were high and over long intervals in late fall and winter.

Results with sediment collectors of various types have been reported (Kleerekoper 1952; Tutin 1955; Thomas 1955; Toyoda et al. 1968), but an assessment of their quantitative value apparently has not been made. In an attempt to determine if sedimentation might be biased by the design of the collectors, a variety of sediment traps (Fig. 2) were operated through three 1-week periods in July at the Glenora station. The collectors ranked in the same order in the three periods (Table 1). The small cylinder caught the greatest amount and the large funnel the least. The amounts of sediment caught in most collectors were of the same order of magnitude, but the results from the small cylinder differed by one order of magnitude from the rest. The large funnel actually may have collected at a rate much closer to that

TABLE 1. Rates of deposition (g m^{-2} day^{-1}) of organic matter in sediment traps of several designs during three 1-week periods in July 1968 at the Glenora station.

Collector		Sedimentation rate			
Design	Diam (*cm*)	July 2–8	9–16	17–23	Total period
Small funnel	12	0.68	0.71	0.40	0.60
Regular funnel	20	0.52	0.38	0.24	0.38
Large funnel	41	–	0.32	0.13	0.26
Small cylinder	5	2.36	2.37	2.92	2.55
Large cylinder	17	0.60	0.66	0.36	0.54

of the regular funnel (the design used throughout the study), because some loss was noted from the surface of the large funnel during its retrieval. The regular funnel collected amounts in each week close to the average for the others excluding the small cylinder, which was probably the only grossly inaccurate collector.

The content of organic matter in detritus was estimated by ashing dried subsamples at 600 C for 0.5 hr. The caloric value of the detritus was estimated with a Phillipson micro-bomb calorimeter (Phillipson 1964) calibrated with benzoic acid standards. Difficulty in igniting sediment, even with the addition of benzoic acid, was remedied by adding a known weight of fuel oil with negligible volatility rather than benzoic acid, about 1 mg of oil to a 30-mg sediment pill, which soaked into the pill and guaranteed ignition. Several samples of oil alone were burned to obtain a correction factor.

Respiration of Sediment Cores

Benthic respiration, which included the microbiota and macroinvertebrates, was determined as follows. Water-sediment samples were collected with the corer described previously (Brinkhurst et al. 1969). A layer of mineral oil about 2 cm thick was placed over the water and a sample of water was removed for oxygen analysis through a side-delivery tube. After incubation for 12–24 hr in a water bath, which was within 1 degree C of field temperature, the water was gently mixed and a second sample for oxygen analysis was removed. Entry of external oxygen was negligible because deaerated (boiled and cooled) water maintained its low initial concentration with similar treatment. The volumes of both mud and water in each test core were measured. Two cores were examined in this manner every 2nd week at each station during the spring and summer in 1968 and once during the previous winter.

Respiration rate was calculated as

$$(C_i - C_f) \cdot V \cdot \frac{24}{T} \cdot \frac{1}{0.00157} \cdot \frac{1}{1000},$$

where C_i and C_f are initial and final concentrations of oxygen in mg liter^{-1}, V is volume of water in liters, T is incubation time in hours, 0.00157 is the area in m^2 of the core surface (15.7 cm^2), and the rate is in grams O_2 m^{-2} day^{-1}. The above rate was multiplied by 3.38 to obtain respiration in kcal m^{-2} day^{-1} (Teal 1957).

Production and Respiration of Macroinvertebrates

Methods used to measure production and respiration of macroinvertebrates have been described (Johnson and Brinkhurst 1971b).

Sedimentation

Sedimentation of detritus at Big Bay averaged 5.55 g dried solids m^{-2} day^{-1}, of which 1.91 g (34%) was organic matter. Glenora sediments received 1.48 g solids m^{-2} day^{-1}, with 0.42 g (28%) organic matter. Conway sediments received 1.37 g solids m^{-2} day^{-1}, with 0.36 g (26%) organic matter. For the lake station, because low winter rates as well as some high spring and fall rates were missed, the average import of 0.66 g solids m^{-2} day^{-1} probably is near the actual mean rate for the entire period. About 32% of the total, or 0.21 g m^{-2} day^{-1}, was organic matter in lake detritus.

The percentages of organic matter in the detritus, obtained as the averages for the samples at the four stations, and the confidence limits were:

	Mean	Observations	Confidence limits ($P = .05$)
Big Bay	35.7	34	34.1–37.3
Glenora	30.0	32	28.2–31.8
Conway	27.9	36	25.8–30.0
L. Ontario	34.3	14	30.1–38.5

Sedimented detritus at Big Bay was higher in organic matter than at the other bay stations, probably because of the considerable import of allochthonous detritus in streams and from nearby marshes. The organic content of lake detritus may have been high simply because inorganic siltation was relatively less in open lake waters. The calculated rate of accumulation of bottom muds based on the inorganic fraction in both sediment and bottom mud was 2 mm year^{-1} at Big Bay, compared with 0.5 mm year^{-1} in Adolphus Reach. Greatest sedimentation rates were expected at the Big Bay station because of the relatively larger area of the littoral zone and proximity of rivers.

Seasonal trends in deposition of organic matter (Fig. 3) were only generally obvious; that is, very low rates were characteristic of the period of ice cover and higher rates otherwise. Early spring and fall rates tended to be high. But high rates occurred at the bay stations from time to time during the summer and deposition rates during occasional brief periods in summer were nearly as low as the winter rates. A decreasing trend through the summer was characteristic of the lake station in both 1967 and 1968.

The means and confidence limits of the caloric contents of sedimented detritus collected at the four stations, determined on several samples per station from different seasons, were:

	Mean kcal g^{-1}	Observations	Confidence limits ($P = .05$)
Big Bay	2.94	4	2.30–3.54
Glenora	3.36	7	2.90–3.82
Conway	2.52	5	1.78–3.36
L. Ontario	2.93	4	2.07–3.79

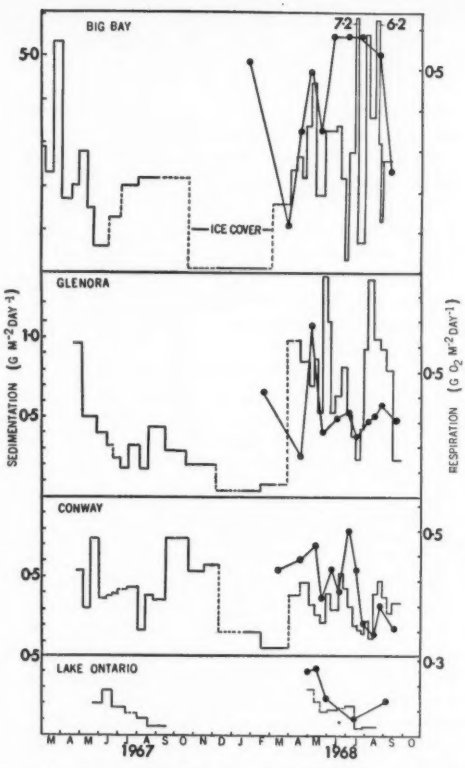

FIG. 3. Rates of sedimentation of organic matter at the four main stations during 1967 and 1968 and respiration rates of sediment cores measured during 1968 (solid circles). Broken lines represent interpolated estimates of missing data.

The mean of all determinations was 2.96 kcal g^{-1}, with confidence limits 2.57 and 3.35 ($\pm 12\%$), and a value of 3 kcal g^{-1} was adopted for general use in the present investigation.

Respiration of Sediments

Corresponding increases and declines in import and respiration were evident at Glenora and Conway in particular, and to a lesser degree at Big Bay and Lake Ontario (Fig. 3). Mean import of organic matter, mean core respiration, and mean temperature at the four stations in 1968 were related (Table 2). However, import and respiration rates were not proportional. That is, the higher import at Big Bay did not lead to benthic respiration one order of magnitude greater than at the other stations. The effect of temperature on respiration probably was confounded with the effect of rate of import of organic matter because no clear relation with temperature was observed *within* each set of observations for each station. When the combined effects of rate of sedimentation and temperature on respiration at each station were examined in multiple-regression analysis, only the Conway regression was significant, in which case only one-half of the variation in respiration could be attributed to these two factors. Variations in the volume of mud and water used in the measurement of respiration had no detectable effect on the results.

TABLE 2. Mean rate of sedimentation of organic matter, mean respiration rate of bottom muds in cores, and mean temperature of incubation during respiration measurement for each of the stations in 1968.

Station	Sedimentation (g m^{-2} day^{-1})	Respiration (g O_2 m^{-2} day^{-1})	Temp (*C*)
Big Bay	3.01	0.35	17.1
Glenora	0.71	0.25	11.8
Conway	0.29	0.22	10.8
L. Ontario	0.28	0.15	9.1

Discussion

A generalized diagram (Fig. 4, after Odum 1968) facilitates description of the flow and use of energy within the benthic community. Imported sediment (IM), consisting of substrate with an active microfloral community, is incorporated with the surface mud. The remaining useful energy becomes generally available to members of the benthic macroinvertebrate community. Because the diverse chemical compounds containing this energy are not entirely susceptible to hydrolysis by the enzymes of macroinvertebrates (Nielson 1962), the mediating role of a diverse microflora with broad spectrum of enzymes is generally recognized.

The microbiota (M) should not be considered a single trophic level. At least four trophic levels should occur in the sediment microbiota: primary producers (only chemosynthetic bacteria in deep sediments), saprophytic microorganisms, and consumers at two trophic levels at least. The significance of chemosynthetic autotrophs in directing energy to consumers in the sediment, in comparison with the import attributable to aquatic and terrestrial photosynthetic autotrophs, is probably small (Macfayden 1963a). Most of the activity of the microbiota may be attributed to the saprophytic microflora, because of the diminishing activity in higher trophic levels,

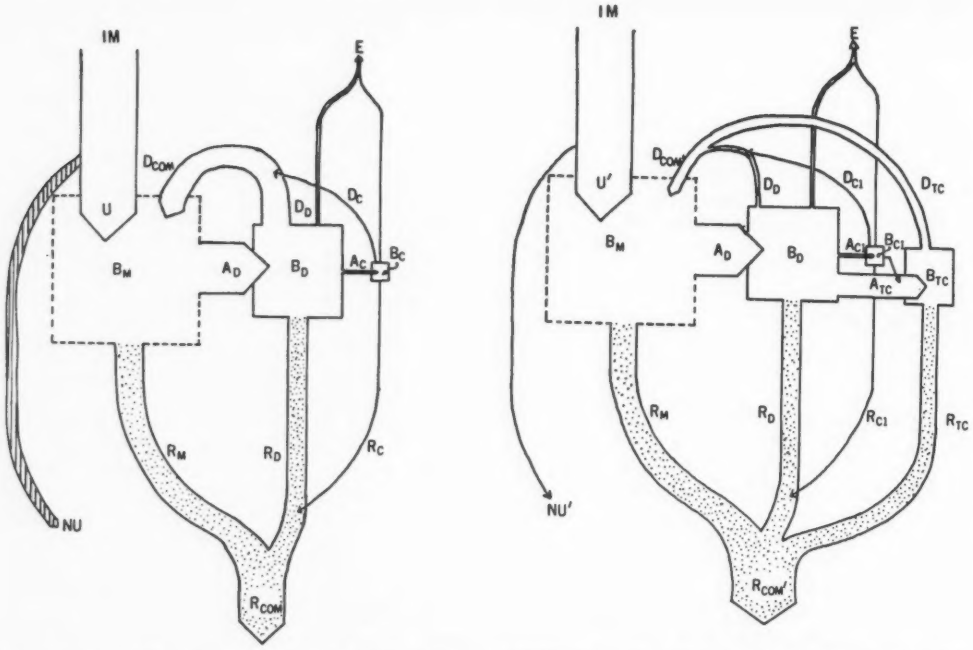

FIG. 4. Models of energy flow through benthic community excluding (*diagram to right*) and including (*diagram to left*) carnivores (benthic fish). Utilized (U) and unutilized (NU) import (IM) are shown at left. In other components, the first letters indicate biomass (B), assimilation (A), decomposition (D), and respiration (R). Subscripts refer to trophic levels including the microbiota (M), detritivores (D), and carnivorous macroinvertebrates (C). Additional explanation and reference to proportions of interest in the model are made in the text. The basis for inclusion of fish to give a complete description of benthic community metabolism is described in text.

that is, on the basis of the Eltonian pyramid in terms of energy flow. In this study the metabolic activity of the microbiota was obtained as the difference between core respiration and respiration of the macroinvertebrate fauna: $R_M = R_{COM} - (R_D + R_C)$.

Community respiration obtained from core respiration is theoretically incomplete because it does not include the metabolic activity of benthic fish that utilize the macroinvertebrate fauna. The top-carnivore trophic level may be added when the following assumptions are made. Only a fraction of the production of macroinvertebrates not already taken by predaceous macroinvertebrates can be ingested by fish, because some export of insects may occur and because production in the form of secretions and exuviae are not usually food items for fish. Natural mortality has been considered negligible compared with predatory mortality in some studies (Lindeman 1941; Welch MS 1967). But exuviae may account for a significant part of net production, for example, about 10% in chironomids (Teal 1957). Therefore, as a *maximum* estimate, no more than about 90% of the production of macroinvertebrates would be ingested by top carnivores. The fish assimilate about 80% of ingested energy and utilize about 75% of assimilated energy in respiration (Ricker 1946; Hayne and Ball 1956). Approximately 20% of ingested energy would be egested, 60% used in respiration and 20% left for growth. Egested material and growth of top carnivores would return (by definition) to the decomposers (Fig. 4). The 60% used in respiration by fish, which is about 50% of the macroinvertebrate production not taken by macroinvertebrate predators, is of primary interest and should be added to core respiration to complete community metabolism ($R_{COM'}$).

The two measures of community respiration, R_{COM} and $R_{COM'}$ (Fig. 4), represent extremes in community metabolism, with the former minimizing and the latter maximizing the use of macroinverte-

brate production by benthic fish. The values of R_{COM} and $R_{COM'}$ would not be widely different because of the high trophic level of the fish.

The macroinvertebrates assimilate energy for their metabolism and growth (A_D) probably to a large extent in the hydrolyzed products of microbial enzymatic action and in the microbes themselves. The detritivores use much of this energy in metabolism (R_D) and use the remainder for growth, including reproduction. Energy in production of detritivores is directed to carnivorous macroinvertebrates (A_C) and to benthic fish, while some is returned to the decomposers (D_D) and some is exported (E) as in the emergence of chironomids in the present study. The proportions that go to benthic fish and return to decomposers depend on the degree of exploitation of the benthos by the fish. Energy assimilated by carnivorous macroinvertebrates (A_C) is similarly transformed in metabolism (R_C), redirected within the community (D_C, A_{TC}), and possibly exported (E).

The energy exported through emergence of chironomids at the Quinte stations was estimated as 17% on the basis of a range of 12–20% found in or calculated from the literature. Welch (MS 1967) found that emerged midges contained 20% of net production. Borutsky's (1939) data provided a value of 12% for *Tanypus* sp. and Teal (1957) reported the export of 20 and 14% of net production for *Calopsectra* (=*Micropsectra*) *dives* and *Anatopynia* spp., respectively, emerging from Root Spring. A value of 19% was obtained for *Tanytarsus* (=*Chironomus*) *jucundus* from the data of Anderson and Hooper (1956), on the assumption that adult chironomids come from prepupal larvae with 1.4 times the energy content (found by Teal in *C. dives*).

Proportions of interest, conveniently obtained from diagrams of energy flow, include the following: First, a is the proportion of energy reaching the benthic community that is utilized, that is

$$a = \frac{R_{COM'} + E}{IM} = \frac{U'}{IM}.$$

Second, b is the proportion of total energy utilized and exported that is attributable to the macroinvertebrates:

$$b = \frac{R_D + R_{Cl} + E}{R_{COM'} + E}.$$

Third, the net growth efficiency of the total macroinvertebrate community is obtained as

$$Eg_c = \frac{A_D - (R_D + R_{Cl})}{A_D} = \frac{P}{A_D}.$$

Mean daily flows and uses of energy at the four stations (Fig. 5) are based on 507 days at Big Bay, 525 days at Glenora and Conway, and 243 days at Lake Ontario (where the period of study was interrupted because winter data were lacking, but where a cautious interpretation may provide useful comparisons with the other stations).

Almost all imported energy was utilized and exported at Glenora, Conway, and the Lake Ontario station. Under usual conditions, excluding bogs, marshes, and swamps, most of the energy of primary production is utilized. Otherwise, organic matter would accumulate exceptionally rapidly (Hairston et al. 1960). The efficiency of use of import (a) was low (0.23) at Big Bay (Table 2). Consequently, high organic content of bottom muds would be expected at Big Bay, because the organic content of sedimented detritus was similar, about 32%, at all stations. The relatively high organic content of 28% in Big Bay mud and high fibre content of both mud and sedimented detritus substantiate the low utilization efficiency and also offer an explanation. Probably the allochthonous fibrous material, which comprised much of the import at Big Bay, is mostly resistant to further microbiological attack. In contrast, the utilization efficiency at the Lake Ontario station was high (0.91), most of the organic import probably was autochthonous material as indicated by a scarcity of fibre, and, consequently, the organic content of bottom mud was only 3–4%. However, the organic content of about 16% in Glenora and Conway muds was greater than one might predict from the high utilization efficiencies at those stations. Clearly, data on the total organic content of benthic muds are not indicative of benthic productivity, although they may have other ecological value. In fact, high organic matter content in bottom muds may indicate deficiencies in production at higher trophic levels, that is in macroinvertebrates and their predators.

Examination of the proportion b may be approached as follows, where $1.0 - b$ is the "loss" of energy to the microbiota. This "loss" of about two-thirds of the total energy utilized at Glenora, Conway, and Lake Ontario (Table 2) was the "cost" to the macroinvertebrate community of mediation (and competition?) by the microbiota. Bornebusch (1930) found that soil animals were responsible for up to 25% of metabolic activity of the litter in an oak wood; that is, the "cost" of microbial mediation was at least 75%. Macfayden (1963b) found that soil animals accounted for

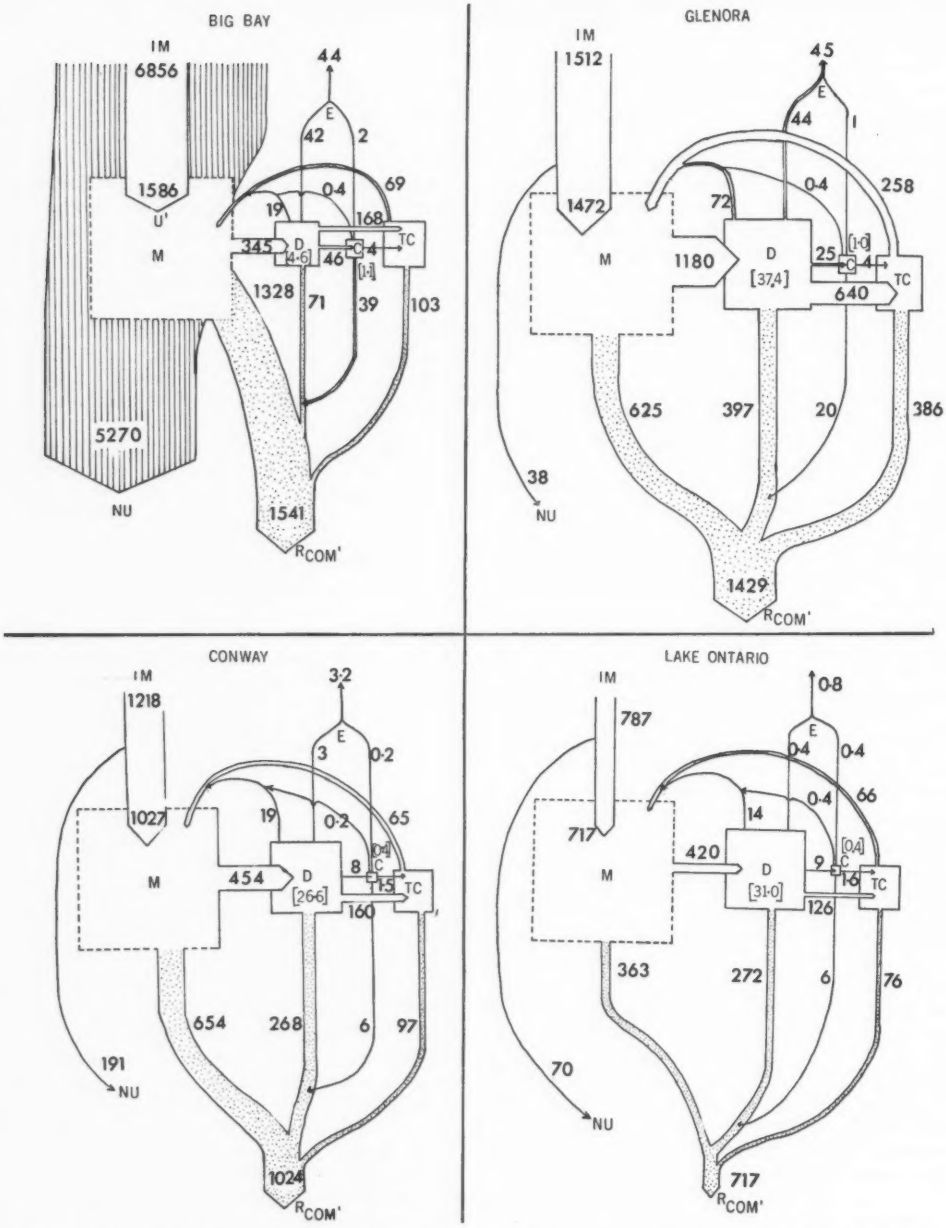

FIG. 5. Average rates of energy flow at the main Quinte stations entered into the model described in Fig. 4. Energy-flow rates are in cal m^{-2} day^{-1}; standing stocks, within brackets, are in kcal m^{-2}.

10–20% of total soil community metabolism. The "cost" of about 90% was greater to the Big Bay macroinvertebrates (where $1.0 - b = 0.9$) than to associations at the other stations. We are tempted to speculate that greater microbiological activity was required to render assimilable energy for macroinvertebrates from the more resistant fibrous import. Conversely, with a relatively lower import of fibrous material, less energy might be "lost" to the microbiota, an idea that receives some support from the highest proportion (0.39) of energy utilized by macroinvertebrates at the Lake Ontario station, where little fibre was observed.

Note that the product of the first two proportions of interest, $a \cdot b$, gives the proportion of imported energy that was utilized and exported by macroinvertebrates. This was only 0.02 for the Big Bay association but was 0.23–0.35 (mean of 0.29) at the other three Quinte stations.

Finally, the proportion of assimilated energy used in growth in entire macroinvertebrate communities, the third proportion of interest (Eg_c, Table 3), averaged about 0.5 over the study period at all stations. The maximum of 0.68 occurred in the Big Bay association, composed mainly of chironomids. The minimum of 0.34 was found in the Lake Ontario macroinvertebrates, in which *Pontoporeia affinis* predominated.

The proportions examined in the preceding section, a, b, and Eg_c, may be used in a concise model of energy utilization and production by macroinvertebrates as a function of total energy import to the benthos. This model should be of value in guiding future research on some functional aspects of macroinvertebrate associations.

Energy utilization (U) of macroinvertebrates is a function of import of energy (IM),

$$U = a \cdot b \cdot IM,$$

where a is the proportion of imported energy used (respired and exported) by the benthic community and b is the proportion of the total energy utilized attributable to macroinvertebrates, that is, respiration and export by macroinvertebrates.

The parameter a probably varies directly with the proportion of autochthonous material in the import. Lower values, as obtained at Big Bay, are to be expected with increasing amounts of allochthonous material. The high mean value of a of 0.9 obtained at the other three stations must be close to the maximum that would be realized in a natural benthic community where some accumulation of energy must occur, albeit at an extremely low rate.

The lower the value of b, the greater is the probable "cost" attributable to mediation by the microbiota. The value of b apparently decreases as the proportion of allochthonous material increases. The shift in primary producers toward relatively more blue-green algae may exert an effect similar to the increase in the proportion of allochthonous import, because these algae often are blown to shore and decompose there. Subsequently, the remaining refractive matter is deposited in the benthic sediments.

Production of macroinvertebrates is a function of energy utilized: $P = c \cdot U$. The parameter c is not obtained directly from growth efficiency, because $U = R + E$, that is, utilized energy is the sum of the respired and exported energy. However, where E is negligible, c is directly available from macroinvertebrate growth efficiency:

$$c = \frac{Eg_c}{1-Eg_c},$$

because $Eg_c = \frac{P}{P + R}$. Therefore, $P = c \cdot U$, and $P = c\ (a \cdot b \cdot IM)$.

The three parameters (a, b, and c) would have to remain constant if production was proportional to import. However, the values of these parameters should decrease through higher levels of import. The product $a \cdot b$ (the proportion of import used by the macroinvertebrate community) decreases as import increases, because of decreases in both a and b. The percentages of organic matter in sedimented detritus were similar in Hamilton Bay (unpublished data) and the Lake Ontario station, yet the percentage of organic matter in the bottom mud was much lower in the open lake, indicating more efficient utilization of imported energy in the lake. Low utilization efficiency, which apparently was related to high import, may occur because of the failure to keep up with import, or, more likely,

TABLE 3. Proportions of interest obtained from energy flow data from the four main stations. The definition and significance of each proportion are given in the text, and the data from which the values were derived are shown in Fig. 5.

Station	Proportion		
	a	b	Eg_c
Big Bay	0.23	0.10	0.68
Glenora	0.97	0.31	0.64
Conway	0.84	0.27	0.40
L. Ontario	0.91	0.39	0.34

because a greater proportion of the import is not readily utilizable. The latter explanation applies to Big Bay with its natural allochthonous import and probably to Hamilton Bay as well. Probably c decreases at levels of import sufficiently high that the environment is substantially altered through the use of that import. Low dissolved oxygen and high concentrations of waste products lead to increased metabolic costs. For example, more time and energy must be devoted by the animal to respiratory activity (Alsterberg 1922; Brundin 1951). Theoretically at least, c may be lower at very low import levels because of the extra activity per unit food obtained in a sparse food supply.

The compound effect as all three values decrease should be emphasized, because a small reduction in each parameter produces a great reduction overall (if $a \cdot b \cdot c = .50 \times .33 \times .67$ and each is reduced by 20%, the product is halved).

Finally, at extremely high levels of import, c may become critically low and production of some or all species may cease altogether. Critically low growth efficiencies have not been defined but they may be related to the development time of a species in relation to abiotic limnological events, prolonged exposure to predators, and other factors.

Changes in the species composition of the community (to so-called tolerant species) would tend to counteract the increasing severity of the environment probably through selection of species able to maintain high growth efficiencies. Also, species may be selected to maintain the community value of b, that is, to minimize the "loss" to mediating microflora. This would be a useful rationale in the study of succession in eutrophication in that it provides testable hypotheses.

In summary, the graphical relation of production of macroinvertebrates to energy import, in view of the probable changes in the parameters, is an inverted "U" intersecting the abscissa (import) at the origin and again at extremely high energy import. The maximum macroinvertebrate production (which may or may not be an optimum, for example, for fish production) or any other level of production eventually should be predictable at a given site from data on import. Import of energy to bottom sediments is related, in part, to primary productivity and the latter to nutrient loadings, and, finally, to the influence of man. In time it should be possible to develop and test models applicable to the Great Lakes that would include nutrient loadings, primary production, energy import to the benthos (with modification of the benthic environment; for example, oxygen deficits), and production and diversity in benthic macroinvertebrates as well as other consumers.

Acknowledgments

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Surges of Winter Flounder, *Pseudopleuronectes americanus*, into the Intertidal Zone

A. V. TYLER

Fisheries Research Board of Canada
Biological Station St. Andrews, N.B.

TYLER, A. V. 1971. Surges of winter flounder, *Pseudopleuronectes americanus*, into the intertidal zone. J. Fish. Res. Bd. Canada 28: 1727–1732.

Movement of winter flounders, *Pseudopleuronectes americanus* (Walbaum), into the intertidal zone was observed by underwater television situated in a herding fence. Flounders enter the intertidal zone with the rising tide. Peak movement occurs 2–2.5 hr after low tide. Movement generally falls off 3.5 hr after tide begins to rise. Flounders occupy the area for 6–8 hr and then surge back to the sublittoral 2.5–0.5 hr before the next low tide. Small flounders move with the same synchrony as large flounders, but tend to make short movements up and down the beach, with net movement away from shore while tide is falling. Large flounders are more strictly directional. Movements of eight species that occurred with the flounder are compared. Because of the scale of the flounder movement, and because of published observations of pleuronectids feeding in the intertidal zone, it is proposed that the intertidal zone is a major feeding area for northern populations of winter flounder. Actions that interfere with productivity in the intertidal zone (oil pollution, tidal impoundments) are likely to reduce productivity of flounder populations.

TYLER, A. V. 1971. Surges of winter flounder, *Pseudopleuronectes americanus*, into the intertidal zone. J. Fish. Res. Bd. Canada 28: 1727–1732.

Un appareil de télévision sous-marine placé à l'intérieur d'un parc a permis à l'auteur d'observer le déplacement des plies rouges, *Pseudopleuronectes americanus* (Walbaum), dans la zone intercotidale. Les plies pénètrent dans cette zone avec le flux. On observe une activité maximale de 2–2.5 h après la marée basse. Le mouvement ralentit 3.5 h après le début du flux. Les plies séjournent dans la zone intercotidale pendant 6–8 h, puis se retirent dans la zone sub-littorale de 2.5–0.5 h avant la marée basse suivante. Le mouvement des petites plies est synchronisé avec celui des grosses, mais les premières ont tendance à monter et descendre le long de la plage, avec résultante vers le large pendant le reflux. Les grosses plies ont par contre des mouvements nettement orientés. L'auteur compare les déplacements de huit espèces de poissons accompagnant les plies. L'amplitude des déplacements, ainsi que les observations déjà publiées sur l'alimentation des pleuronectidés dans la zone intercotidale, nous portent à croire que cette zone est un important territoire d'alimentation des populations nordiques de plies rouges. Tout ce qui nuit à la productivité de la zone (pollution par l'huile, construction de digues) est de nature à diminuer le rendement des stocks.

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THE aim of this study was to find when the numbers of winter flounder, *Pseudopleuronectes americanus* (Walbaum), maximized in the intertidal zone in relation to time of high and low tide. The work was done in Passamaquoddy Bay, N.B., as part of a project to determine flounder densities, predation rates (calories per day-acre), and relative importance of the intertidal zone to flounder production as compared with the shallows of the subtidal regions. Though several good studies have been published on movements of tidepool fishes (Gibson 1969), little information is available on movement of fishes that occupy the area only during high tide. Such fishes are frequently the young of commercially utilized species (Merriman 1947). The intertidal zones of coarse beaches may be of little importance to fish production (Merriman 1947), whereas beaches with finer sediments can be areas of major predation by fish (Hancock and Urquhart 1965).

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Methods

Flounders were observed with underwater television. The camera was a 10MHZ BW model with vidicon giving usable pictures with 0.1 ft-c reflected light (Cohu Electronics, San Diego). The camera was placed 600 ft

(184 m) from the high-water mark (at the low-water mark for spring tides). It was operated with a 729 line synchronizing generator, and a remote lens control module housed in a shed at the high-water mark.

A herding fence for fish moving either toward or away from shore was made of two nets each 100 ft (30.5 m) long and 4 ft (1.2 m) high with 2-inch (5-cm) mesh. The ends of one net were staked 88 ft (27 m) apart on a line running at right angles to the beach. The net was then pulled into a V-shape, with 50-ft (15-m) arms meeting at an angle of about 120 degrees. The second net was set up as a mirror image of the first, so that a double funnel, or hour-glass shape, was formed. There was a 3-ft (.92-m) gap between the points of the V's (the narrows of the funnel). The distal ends of the two nets formed 50-ft (15-m) gaps (the mouths of the funnel). Any fish moving along bottom towards or away from shore between the ends of the two nets were herded over the next 44 ft (13 m) to the 3-ft (.92-m) gap between the nets. The TV camera was positioned over the 3-ft (.92-m) gap so that the entire width of the gap could be observed on the monitor. The camera and narrows of the fence were far enough from the high tide mark so that, at the extreme monthly tidal amplitude, the camera was just out of water at low tide, and at the minimum monthly amplitude it was under 6 ft (1.8 m) of water at low tide.

The TV monitor was observed intermittently from June to October 1970 (508 half-hour periods). The numbers of half-hour intervals at different tidal phases during which fish were counted are given in Table 1. The paucity of observations for intermediate depths with falling tides was due to stormy weather conditions and equipment failure during the 2 or 3 days per month that this tidal phase could be observed. Fish were recorded as moving toward or away from shore. A fish that moved into view, turned, and then swam back in the direction from which it had come was not counted.

Observations were made only during daylight hours, early morning to dusk. Artificial lighting was not used.

TABLE 1. Number of half-hour intervals of observation under various tidal conditions.

Low tide depth at camera (*ft*)	Half-hour interval											
	1	2	3	4	5	6	7	8	9	10	11	12
Before low tide												
0–1.9	0	7	8	9	8	9	9	8	8	8	8	8
2–3.9	1	1	1	1	1	1	1	0	0	0	1	1
4–5.9	15	13	15	15	15	14	14	14	14	13	12	14
After low tide												
0–1.9	0	0	4	7	6	6	7	8	8	8	7	7
2–3.9	3	3	5	6	6	6	6	6	6	6	6	5
4–5.9	11	11	11	10	10	10	9	8	8	8	7	6

In October 1970, 1-hr recordings were made on video tape with a Sony Video Recorder (model EV-200) during times of peak movement as flounders entered the intertidal zone on 2 days and as they left on another day. Fish lengths were taken on playback with the picture stopped. During an 8-day period in the first half of October, fish passing the camera were recorded in two size-groups (those greater than 10 cm and those less than or equal to 10 cm length) to see whether different-size flounders moved at different times. A grid was drawn on the monitor screen with measurements calibrated to distances on the mud–gravel bottom. Sizes estimated on the monitor screen depended on the fact that the flounder rarely moved more than a few centimeters off bottom when they swam (diver's observation).

Results

Synchrony of Flounder Migration and Tide Direction

On the average, 135 winter flounders moved past the camera in a 12-hr tidal cycle. The mean numbers of flounders that moved towards and away from shore per half-hour interval are given in Fig. 1. The mean was calculated by dividing the total number of flounders that moved past the camera during, say, the first half-hour period after high tide, by the number of days on which this interval was observed. As the tide went through its monthly cycle from spring to neap, water depth at low tide varied at the camera site from 0 to 6 ft (1.84 m). Data in panel A were recorded when water depth at low tide was less than 2 ft (0.61 m) (spring tides); in panel B, when water depth was between 2 and 4 ft (1.22 m); in panel C, when water depth was between 4 and 6 ft (1.84 m) (neap tides). There are no data for the half-hour period before low tide and 2 half-hour periods after low tide in panel A, because the camera lens was out of water at these times and could not be operated effectively.

There was little difference in synchrony of movement among flounders with change of depth at low tide. For all data combined regardless of depth, 68% of the flounders moved up the beach in a 2-hr period beginning 1 half-hour after low tide (89% in a 3-hr period). Seventy percent moved away from shore in a 2-hr period ending 1 half-hour before low tide (88% in a 3-hr period). Thus, about 70% of the flounders that moved into the intertidal zone occupied it for 7 hr of the 12-hr cycle. There was little movement in either direction 1 half-hour on either side of low tide and little movement 2.5 hr before or after high tide. Thus, flounders entered the intertidal zone in a surge. While the tide was rising, very few flounders left the intertidal zone. While the tide was falling, very few entered.

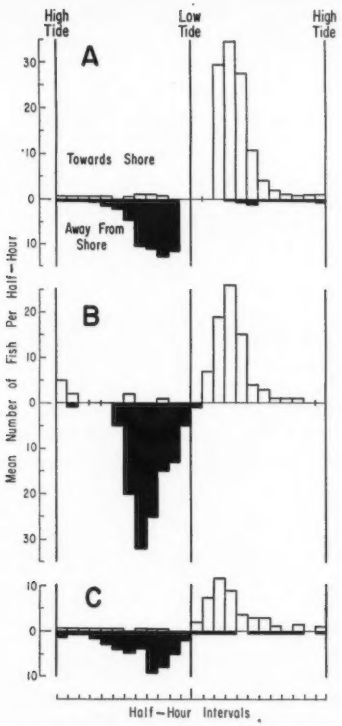

FIG. 1. Mean numbers of flounders moving towards and away from shore per half-hour interval with reference to the 12-hr tidal cycle. Panel A is data from days with low tide depths of 0–1.9 ft at camera location; panel B: when low tide depths were 2–3.9 ft at the camera; panel C: when low tide depths were 4–6 ft at the camera.

COMPARISON OF MOVEMENT OF LARGE AND SMALL FLOUNDERS

The length-frequency distributions from video tapes show two size-groups of fish, one 4–15 cm long and one 25–49 cm long (Fig. 2). Each of the three samplings showed about the same length distribution with the peculiarity that intermediate sizes (11–25 cm) were absent.

For the fish recorded into two size-groups (greater than, or less than, or equal to 10 cm) during October, mean number of flounders per half-hour, swimming either towards or away from shore, was expressed as a percentage of the mean number of flounders swimming past the camera per 12-hr tidal cycle (Fig. 3). The mean number of flounders per tidal cycle was calculated by proportion as the total number of flounders observed divided by the total number of half-hour periods of observation, multi-

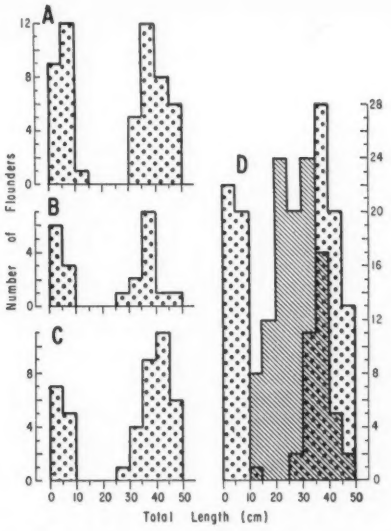

FIG. 2. Length-frequency histograms of flounders that swam past the camera during 1–hr periods at times of peak movement on three separate days in October (graphs A, B, C). Graph D is A, B, C, combined, with a superimposed sample of flounders from the deep part of the bay in early March (diagonal shading).

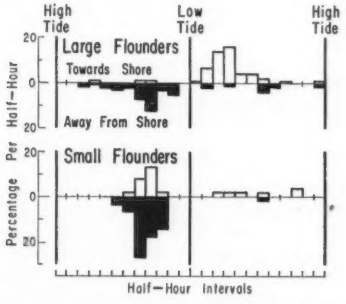

FIG. 3. Mean numbers of flounders per half-hour, moving towards and away from shore, expressed as a percentage of the mean number of flounders moving past the camera per a 12-hr tidal cycle. Upper panel is from fish greater than 10 cm, lower panel from fish less than or equal to 10 cm.

plied by the number of half-hour periods in a 12-hr cycle (24). Peak movement away from shore for both large and small flounders occurred $1\frac{1}{2}$–$2\frac{1}{2}$ hr before low tide. The large flounders did not move out of the intertidal zone ahead of the small flounders. It might be expected that the smaller fish would

remain longer in more shallow water and so move out after the large fish did. One difference between movement of large and small flounders was evident from Fig. 3. Though very few large flounders showed movement towards shore immediately before low tide, many small flounders moved both towards and away from shore, with greater percentages moving away during any half-hour interval. Apparently, while the large flounders moved out, there was little turning and reversing direction. However, the small fish likely moved back and forth past the camera with a net or residual movement out of the intertidal zone during falling tide.

Other Fishes that Occupied the Intertidal Zone

Eight species of fish other than winter flounder were seen under the camera during the 204 hr of observation. These species and the number of times they were seen (probability of seeing the same individual more than once must be high) are as follows: Atlantic herring (*Clupea harengus harengus*), many schools; Atlantic tomcod (*Microgadus tomcod*) and young Atlantic cod (*Gadus morhua*), 423; Atlantic cod 30–40 cm long, 6; longhorn sculpin (*Myoxocephalus octodecemspinosus*), 113; little skate (*Raja erinacea*), 52; ocean pout (*Macrozoarces americanus*), 32; sea raven (*Hemitripterus americanus*), 28; shorthorn sculpin (*Myoxocephalus scorpius*), 1. Also, an American lobster (*Homarus americanus*) was seen once.

For each of the above fishes, except herring and shorthorn sculpin, the mean numbers of fish moving towards and away from shore in each half-hour interval before and after low tide are expressed in Fig. 4 as a percentage of the mean number of fish (of the species) that moved past the camera pre 12-hr tidal cycle. Herring moved past the camera too quickly to be counted. Longhorn sculpin tended to move with the tide, as did the flounders, but the movement was not so strongly limited to a few hours before and after low tide. The sea raven pattern was similar to the sculpin pattern. Tomcod and young Atlantic cod, which could not always be distinguished from one another on the TV monitor, moved up and down the beach, either with or against tidal current, except during the hour either before or after high tide. Ocean pout and little skate showed no synchrony between their movements and tidal stage.

All species occurred throughout the observation period (June 24–October 16) except ocean pout, which were more frequently seen in early summer. All but four of the ocean pout were observed between June 24 and August 1.

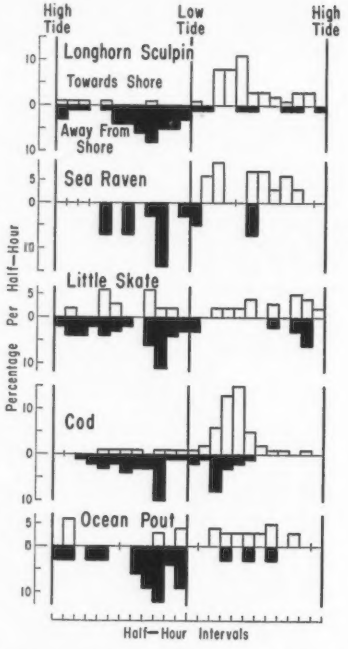

FIG. 4. Mean numbers of fish per half-hour, moving towards and away from shore, expressed as a percentage of the mean number of the species moving past the camera per 12-hr tidal cycle.

Discussion

The unusual length-frequency of winter flounders in the shallows in Passamaquoddy Bay was previously discussed by McCracken (MS 1954, 1963), who found that in summer, large mature flounders and small immature flounders occurred at depths of less than 5 fath (9 m). Intermediate-size fish were scarce at these depths but present in deeper waters (6–10 fath, 11–18 m).

To ascertain that the unusual length-frequency distribution reported here was actually the same phenomenon reported by McCracken, rather than lack of recruitment to the population during a period of years, a sample of 112 flounders was taken by otter trawl from the deep area of Passamaquoddy Bay and was measured in early March 1971. This sample should be representative of the entire flounder population in the Bay, since the flounders are concentrated in the deepest areas when Bay temperatures are at their minimum. The superimposed histogram in Fig. 2 shows that intermediate sizes were strongly represented in the Bay.

McCracken found that this distribution of flounders held for Passamaquoddy Bay and Pubnico Harbour, Nova Scotia, but not in the Northumberland Strait (Gulf of St. Lawrence) or Long Island Sound where both intermediate and large fish occurred in the deeper water. McCracken gave evidence that the lack of intermediate and large flounders in the shallows of the latter areas was due to water temperature greater than 15 C. Intertidal water in Passamaquoddy Bay, where large flounders occurred together with small flounders, generally did not rise above 14 C. The absence of intermediate-size flounders in Passamaquoddy Bay was related to light intensity. In laboratory experiments, McCracken showed that flounders of intermediate size avoided even low light intensities, but that the smallest and largest fish did not. He also showed that the lack of avoidance in the large fish was associated with onset of maturity.

The vigorous movement of winter flounder into the intertidal zone doubtless has adaptive significance. It is unlikely that they escape from predators by moving into shallows. By concentrating themselves in a small region with little cover the fish possibly increase their chances of being eaten. I have seen both great blue heron and osprey take fish in the intertidal areas of Passamaquoddy Bay. Furthermore, seals frequent shallow areas in the Bay. Sea raven (*Hemitripterus americanus*), a fish known to prey on winter flounder (Tyler MS 1968), is present in both shallow and deep coastal areas.

It is more likely that the flounders enter the intertidal zone to feed. Throughout much of the range of the winter flounder the zone holds dense beds of the maldanid polychaete, *Clymenella torquata* (Mangum 1964). Densities of over 1000 worms per square meter have been recorded intertidally in Brandy Cove (Rowe MS 1970). Maldanid polychaetes are a principal prey in other areas of Passamaquoddy Bay (Tyler MS 1968). Winter flounder feed intertidally on soft-shell clams (*Mya arenaria*), and these are largely restricted to the intertidal zone in Passamaquoddy Bay (Newcombe 1936). Over half of the flounders caught by set-seine (a V-shaped net with mouth opening towards shore) in the intertidal zone near Brandy Cove in April 1949, had young-of-the-year clams in their stomachs (Medcof and MacPhail 1952). One fish had 315 clams in its stomach. Two-thirds of the flounders collected contained nipped-off siphon ends of older *Mya*.

Evidence for massive occupation of the intertidal zone by pleuronectid fishes has been published previously. A high percentage of the plaice (*Pleuronectes platessa*) along the shore of Loch Ewe, Scotland, entered the intertidal zone (Edwards and Steele 1968). Plaice densities in shallow water at low tide were consistently higher than densities at high tide, indicating that the fish concentrated near the water's edge at low water and then spread into the intertidal zone at flood tide. Hancock and Urquhart (1965) found that the flounder (*Platichthys flesus*) ate quantities of cockles (*Cardium edule*) in the Burry Inlet, South Wales. On the average, there were 120 young cockles per stomach in fish caught in set-seines on falling tides.

Smidt (1951) showed that the large intertidal flats of the Danish Waddensea were important feeding grounds for plaice (*Pleuronectes platessa*) and other species of fish. The intertidal areas held a much greater biomass of fish-food organisms than did the tidal channels that drained the flats. Smidt suggested that the biomass was reduced in the channels because of continuous predation by a variety of organisms including fish, crabs, shrimps, and starfish.

Since it is established that pleuronectids feed heavily in intertidal areas, and since this study has shown that winter flounders occupy the intertidal zone for 6–8 hr of the 12-hr tidal cycle in daytime, it is likely that the intertidal zone is a major feeding area for inshore populations of the species. Interference with the intertidal zone as an area of natural production, either through pollution (e.g., oil spills) or tidal power projects, will probably result in substantial decreases in flounder production.

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Production Potential of a Seaweed–Lobster Community in Eastern Canada[1,2]

R. J. MILLER,[3] K. H. MANN

Fisheries Research Board of Canada
Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

AND

D. J. SCARRATT

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

MILLER, R. J., K. H. MANN, AND D. J. SCARRATT. 1971. Production potential of a seaweed–lobster community in eastern Canada. J. Fish. Res. Bd. Canada 28: 1733–1738.

An energy-flow diagram for the bottom community in the seaweed zone of St. Margaret's Bay, Nova Scotia, has been constructed, using previous estimates of seaweed production and observations on animal biomass and respiration rates. Annual seaweed production exceeded the annual consumption by herbivores by a factor of more than 10 (7000 vs. 572 kcal/m^2 per year). This fact, plus observations of physical processes in the seaweed zone, indicate that most of the seaweed production is exported as particulate matter in suspension. Production rates of the American lobster (*Homarus americanus*) prey species exceed lobster ingestion rates, also by a factor of more than 10 (84 vs. 6 kcal/m^2 per year). The difference is presumably consumed by lobster competitors. The available information suggests lobster production in the seaweed zone could be increased by reducing predation and competition for food, together with an increase in suitable shelter.

MILLER, R. J., K. H. MANN, AND D. J. SCARRATT. 1971. Production potential of a seaweed–lobster community in eastern Canada. J. Fish. Res. Bd. Canada 28: 1733–1738.

Les auteurs ont construit un diagramme de transport énergétique pour la communauté benthique de la zone des algues de la baie Sainte-Marguerite, Nouvelle-Ecosse, en utilisant des données antérieures de production d'algues et des observations relatives à la biomasse animale et aux taux respiratoires. La production annuelle d'algues est 10 fois supérieure à la consommation par les herbivores. Ceci, en plus des données physiques du milieu, indique que la majeure partie de la production d'algues est disséminée sous forme de matière en suspension. Le taux de production des proies du homard américain (*Homarus americanus*) est, lui aussi, 10 fois supérieur au taux d'ingestion par cette espèce. La différence est probablement utilisée par des espèces compétitrices. Les données actuelles suggèrent que la réduction des prédateurs et des compétiteurs, accompagnée d'une augmentation du nombre d'abris appropriés, favoriserait la production du homard dans la zone des algues.

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A well-defined community on the rocky shores of eastern Canada is the sublittoral kelp zone, with its characteristic herbivores, such as sea urchins and periwinkles, and a variety of carnivores, of which the most valuable is the American lobster (*Homarus americanus*). A start has been made on the analysis of the trophodynamics of this system, with a view to determining the productivity of the seaweeds and the potential for increasing lobster production. This paper considers questions of economic importance, such as: Are the dynamics of the system such that lobster productivity is limited by productivity of the lower trophic levels? Could lobster production be enhanced? Could seaweeds be harvested without adversely affecting the yield of lobsters?

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[2]Bedford Institute Contribution BI 272.

[3]National Research Council of Canada Postdoctoral Fellow. Present address: Fisheries Research Board of Canada, Biological Station, St. John's, Nfld.

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Animal population production may be limited by recruitment, food energy, food quality, predators, parasites, or space. Not all of these factors are easily quantified and not all are equally important. Food energy, expressed as production of prey, is frequently considered to be important; therefore, this is a logical start in studying what limits the size of a particular animal population. However, the usual method of estimating population production and consumption requires data on population dynamics, growth, metabolic rates, and assimilation efficiency and is therefore laborious. A recent paper by McNeill and Lawton (1970) demonstrated a correlation between population respiration and population production for a wide range of poikilotherms, so the opportunity has been taken to infer the productivity of the invertebrates in the food web from a combination of in situ measurements of their respiration and population biomass estimates. The confidence limits of such estimates are rather wide, but do not affect the major conclusions.

Methods

Biomass

Observations on the biomass of seaweed and of the major groups of invertebrates in the seaweed zone in St. Margaret's Bay, Nova Scotia, were made by scuba diving in the summer of 1968. Samples were taken on 24 transects laid out at right angles to the shore from randomly chosen points. Ten major seaweed zones were recognized (Mann 1971) and on each transect the zones that were well represented were sampled with $\frac{1}{2} \times \frac{1}{2}$-m quadrats. The average length of a transect was 369 m, the average maximum depth on a transect was 20 m, and the total number of quadrat samples was 165. The divers removed the macrophytes and macroinvertebrates from each quadrat, and identified, counted, and weighed them. The mean biomass per m^2 for each taxon was weighted according to the mean width of each seaweed zone to provide an estimate of the mean biomass per m^2 for the whole seaweed zone of the bay. The methods and results of the macrophyte sampling are given in more detail in Mann (1971).

The lobster biomass was estimated indirectly, since special techniques are required to sample lobster populations (Scarratt 1968). Two parameters were used: (1) the best estimate of average stock of legal-sized lobsters in this part of Nova Scotia, taken from Wilder (1965); (2) the ratio of legal-size stock (Wilder 1965) to total stock (D. J. Scarratt unpublished data) in Northumberland Strait.

Biomass was converted to calories using the data in Brawn et al. (1968) or by using ash-free dry weight/live weight ratios (Thorson 1957) times a constant of 5 kcal/g ash-free dry weight.

Respiration Rates

The respiration rates (in ml O_2/animal per day) of six species of invertebrates important in the diet of lobsters were measured in situ in St. Margaret's Bay at depths of 2–4 m in August 1970. One or more specimens of a species were gently transferred to wide-mouthed bottles, which were capped and left on the bottom for about 6 hr; 5–8 bottles, each with a different mean size of animal, were used for each species. Rates of oxygen consumption were determined from the differences in dissolved oxygen concentration in experimental and control bottles.

In addition, the respiration of sea urchins was studied more intensively because of their importance in the seaweed community. Measurements were made in the laboratory at 2-month intervals at a temperature and salinity close to that in the field. Animals were held in the laboratory only a few days before the experiments. The procedure was similar to that used in the field. Lobster respiration rates were taken from McLeese (1964).

In extrapolating to field conditions, the values obtained in experimental bottles were taken to be fairly representative of the normal activity of the animals, all relatively sluggish in their habits.

To extrapolate from the temperature at which measurements were made to the seasonal range of temperatures we assumed a Q_{10} of 2.05. This value was obtained by fitting a regression line of respiration on temperature to published data for 14 species of marine temperate poikilotherms (R. J. Miller unpublished data). Seasonal temperatures in St. Margaret's Bay were taken from Sharaf El Din et al. (MS 1970).

Temperature was assumed constant for 2 months at a time. The annual respiration rate for each population (R_{ann}) was calculated by summing the 2-monthly values (R_i) as follows:

$$R_{ann} = \sum_{i=1}^{i=6} R_i$$

$$R_i = 61 R_d \times B \times 4.83 \times 10^{-3},$$

where R_d is ml O_2 consumed/g animal per day, derived from the observed respiration rate/g live weight for an animal of average size in the population, and corrected for the appropriate environmental temperature; B is population biomass in g/m^2, and 4.83×10^{-3} converts from ml O_2 to kcal.

Production Rates

Invertebrate production was estimated from a modification of the regression of log population production on log population respiration given by McNeill and Lawton (1970). The modification was provided by using more recent data on fish populations in the River Thames (Mann et al. 1971). The new coordinates, $P_{ann} = 137$ and $R_{ann} = 671$, represent the combined populations of roach and bleak, and replace the points given in McNeill and Lawton (1970) for roach, bleak, dace, perch, and gudgeon. The revised regression equation is:

$$P_{ann} = 0.6440\ R_{ann}^{0.8517},$$

where P_{ann} is annual population production, and R_{ann} is annual population respiration, both in kcal/m² per year.

Seaweed production was estimated by punching holes in blades of *Laminaria* and *Agarum* and measuring the growth of new tissue from the base of the blade (Mann 1972). Growth in length was converted to growth in biomass by means of length/weight regressions and the annual value for the mean production/biomass ratio was applied to the biomass observed in the survey.

Food Consumption

Assimilation efficiency was assumed to be 80% for carnivores and 60% for herbivores. The former is the value given by Winberg (1956) as a reasonable average for all fish and the latter is an average over season and animal size for *Strongylocentrotus droebachiensis* feeding on *Laminaria longicruris* (R. J. Miller unpublished data). Food energy C was calculated from respiration R and production P (both in kcal/m² per year) as

$$C = \frac{P+R}{0.8} \text{ for carnivores and}$$

$$C = \frac{P+R}{0.6} \text{ for herbivores.}$$

Species of Food Consumed by Lobsters

Stomach contents of lobsters collected in the Northumberland Strait were analyzed for food composition by volume (D. J. Scarratt unpublished data). Volumes of each species were converted to calories by assuming a specific gravity of 1 and using the calorific values given by Brawn et al. (1968).

Results

Sea urchins (*Strongylocentrotus droebachiensis*) clearly dominated the invertebrate biomass in the seaweed zone (Fig. 1). Periwinkles, primarily *Littorina littorea*, made a significant contribution to herbivore biomass, as did the mussels. The biomass of several groups not included in Fig. 1 was recorded in the survey of the seaweed zone, but none exceeded 1 g/m² and all totalled only about 3 g/m². Small animals, such as amphipods and polychaetes and the active rock crabs, may not have been sampled quantitatively. Note that "mussels" in Fig. 1 refers to both the blue mussel (*Mytilus edulis*) and the horse mussel (*Modiolus modiolus*).

A figure for probable density of commercial-size lobster stock in St. Margaret's Bay (interpolated from Wilder (1965)) is 1.8 g /m². The mean estimate

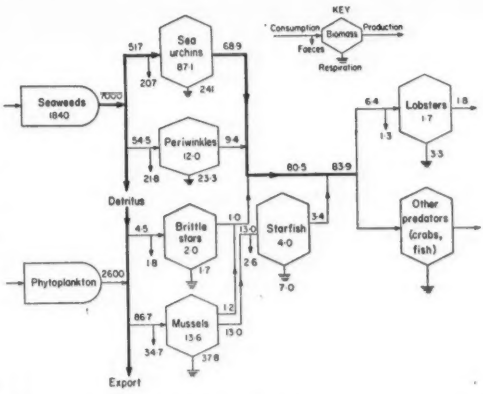

FIG. 1. Energy flow through the bottom community in the seaweed zone of St. Margaret's Bay, Nova Scotia. Units are kcal/m² per year, except for biomass, which is in kcal/m². Phytoplankton production figure from Platt (1971).

of total stock in Northumberland Strait off Richibucto, N.B., is 5.1 g/m², and in that area the commercial stock is thought to average 1.7 g/m². So the best estimate for the total stock of lobsters in St. Margaret's Bay is $1.8 \times \frac{5.1}{1.7} = 5.4$ g/m², or 1.7 kcal/m² (Fig. 1).

The results of respiration experiments are summarized in Table 1 and population respiration energy is given in Fig. 1 under the symbol suggested by Odum (1967) to indicate an energy sink. The value for sea urchin respiration in Fig. 1 is based on the bimonthly laboratory measurements.

Production and consumption energies have approximately the same ratio to biomass for each of the animal groups considered (Fig. 1), so the sea urchins have the largest values for these parameters as well. Consumption and production of the herbivores totals 663 and 93.5 kcal/m² per year, respectively. The consumption and production rates of the predators for which there is information are small relative to herbivore rates. Fish and crab predators are given a compartment in Fig. 1, since they are certainly important in energy transfer through this community, but no data are available.

The biomass estimate including all species of seaweed averages 4.1 kg/m². Paine and Vadas (1969) gave data on calorific value for 15 species of phaeophytes, which average 460 kcal/kg wet weight. Hence, the biomass at the time of the survey may be expressed as 1886 kcal/m². The ratio of production to biomass differs markedly according to species and depth, but the *Laminariales* con-

TABLE 1. Respiration rates, expressed as oxygen consumption per gram per day, and correlation coefficient of regression of log respiration on log weight.

Species	Temp (*C*)	Mean size (*g*)	No. observations for regression	Correlation coefficient *r*	Oxygen consumption (*ml/g per day*)
Lobsters, *Homarus americanus*	12	450	14	–	0.514±0.095[a]
Starfish, *Asterias vulgaris*	17	5.0	5	0.99	1.14
Mussels, *Mytilus edulis* and *Modiolus modiolus*	17	2.2	8	0.59	0.876
Brittle stars, *Ophiopholis aculeata*	17	1.0	5	0.75	0.364
Periwinkles, *Littorina littorea*	17	1.4	6	0.95	0.600
Sea urchins, *Strongylocentrotus droebachiensis* (field)	17	8.3	6	0.99	0.464
S. droebachiensis (lab.)	2	8.3	18	0.98	0.284
	4	8.3	20	0.97	0.400
	11	8.3	18	0.99	0.531
	17	8.3	20	0.99	0.676
	8.5	8.3	18	0.99	0.417
	7.5	8.3	11	0.99	0.371

[a] From McLeese (1964).

stitute more than 80% of the biomass and their P/B ratios have been determined for a variety of environmental conditions. The problem of applying these to the bay as a whole has not yet been resolved, but a P/B ratio between 3 and 5 is a conservative estimate. Hence, production in the seaweed zone is likely to be at least 7000 kcal/m^2 per year[4].

The production of lobster prey species and probable rates of consumption of these species by lobsters are compared in Table 2. The energy contribution of each prey species identified in the stomachs of lobsters from the Richibucto area (estimated from the calorific value of each prey and its relative abundance) are included, since this (D. J. Scarratt unpublished data) is the most complete lobster food-habit study to date. However, there is evidence that lobster stomach contents reflect the relative abundance of prey species in the habitat. The percentage of lobster stomachs in which various species prey were found in a study off the Prince Edward Island coast (D. J. Scarratt unpublished data) are: mussels 67%, rock crabs 50%, polychaetes 42%, periwinkles 36%, sea urchins 23%, and starfish 5%. A study by Squires (1970) in western Newfoundland also includes percentage occurrence of prey; polychaetes 37%, rock crab 27%, periwinkles 22%, sea urchins 4%, mussels 4%, starfish 3%, and brittle stars 1%.

[4]Footnote added in proof: The production in the seaweed zone is now estimated at more than 17,000 kcal/m^2 per year (Mann 1972).

Because of the above evidence, the estimated consumption of each prey species by lobsters is given in the same proportion as production of these species (Table 2). In calculating these proportions the production of mussels is reduced by the energy ingested by starfish, since starfish are a competitor of lobsters. Comparison of columns *c* and *d* in Table 2 shows that prey production far exceeds lobster consumption for each prey species and for the column totals.

As an example of the degree of imprecision involved in the methods used here, confidence limits were accumulated through each major step of the calculations, from daily respiration rates of single animals to the annual production rate of a population (Table 3). The example used, the sea urchin population, includes the respiration rates at a single temperature in the field rather than the laboratory rates. The confidence limits are expressed as 2 standard errors of the mean (2 SE). Note that the data in column 2 are in log form. Since antilog (ln mean + ln 2 SE) = mean × (2 SE) and antilog (ln mean – ln 2 SE) = mean/2 SE, column 3 represents the value by which the mean would be multiplied and divided to get the mean ±2 SE. The upper confidence limit on the estimate of respiration rate at the temperature of measurement is 17% above the mean. The corresponding value for the annual respiration rate is 21% above the mean, the difference being derived from the confidence limits on the average Q_{10}. A further increase in upper limit to 60% above the mean is attributable to the

TABLE 2. (*a*) Percentage composition of the stomach contents of 550 lobsters from Richibucto, N.B.; (*b*) estimated percentage consumption of each type of prey by St. Margaret's Bay lobsters; (*c*) estimated consumption of each type of prey by a lobster population of density 5.4 g/m^2 (= 1.7 kcal/m^2); (*d*) production of each type of prey in St. Margaret's Bay.

Prey	(*a*) % of energy of stomach contents	(*b*) Estimated % consumption by lobsters	(*c*) Estimated consumption by lobsters (*kcal/m^2 per year*)	(*d*) Production by prey (*kcal/m^2 per year*)
Rock crab	28	[a]	–	[a]
Starfish	28	4	0.3	3.4
Sea urchin	10	83	5.2	68.9
Polychaete	10	[a]	–	[a]
Mussel	9	1	0.1	1.1
Periwinkle	–	11	0.1	9.5
Brittle star	–	1	0.1	1.0
Others	15	[a]	–	[a]
	100	100	6.4	83.9

[a]Not sampled quantitatively in St. Margaret's Bay.

TABLE 3. Accumulation of 2 standard errors (2 SE) from the measurement of individual sea urchin respiration to the estimate of population production.

Measurement	ln mean ± ln 2 SE	Antilog 2 SE
Respiration rate at temp of measurement	−0.9136±0.1616 ml O_2/g animal per day	1.17
Mean annual respiration rate	−1.6605±0.1926 ml O_2/g animal per day	1.21
Population respiration rate	4.6663±0.4778 kcal/m^2 per year	1.60
Population production	3.5344±1.6976 kcal/m^2 per year	5.46

confidence limits on population biomass estimates. The final step, introducing the production/respiration relation, brings the upper limit of confidence to 446% above the mean.

Discussion

Calculations of confidence limits on sea urchin production showed the production–respiration plot (modified from McNeill and Lawton 1970) to be the biggest contributor to imprecision. This is not surprising, considering the diversity of animals and habitats represented by the data and the variety of methods used. If future work is conducted using more uniform methods, such as those being developed in the International Biological Programme, some of the previous results may be shown to be unrepresentative of a taxon and may be eliminated from the regression.

The production of seaweeds (>7000 kcal/m^2 per year) far exceeds the ingestion by herbivores (663 kcal/m^2 per year). This figure is not derived entirely from seaweeds, since sea urchins and periwinkles make extensive use of periphyton and small macrophytes, and mussels and brittle stars feed primarily on phytoplankton. The biomass of other benthic herbivores was insignificant, and we know of no pelagic species that might take a measurable fraction of seaweed production. Therefore, it appears that most of the seaweed production is exported from the seaweed beds. In St. Margaret's Bay, at least, the export probably enters the water column as detritus eroded from the seaweed blades by wave action. Large pieces of seaweed are not often seen suspended in the water column or settled on the bottom outside the seaweed zone, and accumulations on the beach are small.

The existence of an excess of seaweed production over herbivore consumption does not mean that seaweed could be harvested without affecting the food web leading to lobsters. If the seaweed were harvested from a large area of bottom, the relatively

immobile herbivores in that area would experience reduced food availability, which would probably limit their production.

The production of lobster prey species (less starfish ingestion) totals 83.9 kcal/m^2 per year (Fig. 1). Comparing this with the ingestion by lobsters, 6.4 kcal/m^2 per year, it appears that food production greatly exceeds that needed to support the lobster population.

There are several possible explanations of why, in spite of this large food production, lobster production is not greater. Not all of the production of prey species would be available to lobsters because some of it occurs in sizes and population densities that they cannot exploit, and because other predators compete for common prey. Moreover, food quality, predators, parasites, space, or recruitment might be limiting.

It is clear that much more research is needed to assess the importance of factors regulating lobster population production. However, as an alternative to waiting for more complete background information, an approach is suggested, based on available data and deductions from ecological principles, for increasing lobster production in a seminatural environment.

A natural area of lobster habitat would be surrounded by a barrier to exclude lobster predators and species competing with lobsters for food. The barrier should not impede water movements unduly since the productivity of seaweeds depends on free flow of water to transport nutrients and remove waste products. The design of the barrier would present problems. Extra lobster homes would be provided in the form of large rocks or pipes (Scarratt 1968; Chittleborough 1970). The area would then be stocked with lobsters at a density higher than is natural, and the resulting productivity determined. Questions requiring detailed study include: grazing efficiencies and productivity of lobsters at different stocking densities; competitive interactions, including cannibalism, between lobsters at high density; effect of removal of competing predators, including the possibility that some prey species not exploited by lobsters might undergo population explosion.

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Some Factors in Susceptibility of Juvenile Rainbow Trout and Chinook Salmon to *Chondrococcus columnaris*

M. P. FUJIHARA, P. A. OLSON,[1] AND R. E. NAKATANI[2]

Department of Biology
Pacific Northwest Laboratories, Battelle Memorial Institute
Richland, Washington 99352, USA

FUJIHARA, M. P., P. A. OLSON, AND R. E. NAKATANI. 1971. Some factors in susceptibility of juvenile rainbow trout and chinook salmon to *Chondrococcus columnaris*. J. Fish. Res. Bd. Canada 28: 1739–1743.

Susceptibility of juveniles of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*) to *Chondrococcus columnaris* disease appeared to be influenced by age of fish, crowding, and water temperature. Rainbow trout of about 1 g and chinook salmon of about 3 g were less susceptible than smaller fish, and tests with chinook salmon suggested that age was more important than weight. When chinook salmon averaging 4.3 g were exposed from July to October to the pathogen in river water, mortality ranged from 1.0% in a trough with 50 fish to 12% in one with 900. Mortality of rainbow trout averaging 2.4 g that were exposed to the pathogen in trough river water averaged 4.7–22% after 4 weeks, at temperatures from 17.2 to 17.8 C; the survivors, however, were generally resistant to subsequent infection, suggesting development of an immune response. Times to 100% mortality of sibling chinook salmon averaging 3.9 g that were exposed for 25 min to about 2.5×10^5 organisms per liter of sterile river water were about 19 hr at 10 C and 8 hr at 22 C. Survival of rainbow trout was higher when they were held at 2.2 degrees C below than when held at 2.2 degrees C above seasonal river temperatures.

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La prédisposition des saumons chinook juvéniles (*Oncorhynchus tshawytscha*) et des truites arc-en-ciel (*Salmo gairdneri*) à l'infection à *Chondrococcus columnaris* semble être fonction de l'âge du poisson, du surpeuplement et de la température de l'eau. Les truites et les saumons pesant environ 1 et 3 g respectivement sont moins susceptibles que les poissons plus petits. Des essais sur les saumons chinook indiquent que l'âge est plus important que le poids. Des saumons exposés à l'agent pathogène en eau de rivière pendant les mois de juillet à octobre ont subi une mortalité allant de 1.0% en vivier de 50 poissons à 12% en vivier de 900. Quatre point sept à vingt-deux pour cent des truites arc-en-ciel d'un poids moyen de 2.4 g, exposées à *C. columnaris* en vivier de température de 17.2–17.8 C, sont mortes au bout de quatre semaines. Par contre, les survivants ont généralement résisté à une infection subséquente, signe de développement d'une immunité. Les temps requis pour que meurent 100% des saumons de la même progéniture, pesant 3.9 g en moyenne et exposés pendant 25 min à des concentrations de 2.5×10^5 organismes par 1 d'eau de rivière stérile, ont été de 19 h à 10 C et 8 h à 22 C. Les truites arc-en-ciel gardées à une température de 2.2 C plus basse que la température saisonnière de la rivière ont mieux survécu que celles gardées à une température de 2.2 C plus haute que celle de la rivière.

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FISHERIES interests and the general public have become increasingly concerned about the possible detrimental effects of elevated river temperature, especially in those rivers that support anadromous salmonids. Fishes infected with *Chondrococcus columnaris* disease are observed in the Columbia River and tributaries during the late spring, summer, and fall, when the temperatures are warm, but rarely during the winter. Serious losses of adult anadromous salmonids attributed to *C. columnaris* disease during summer river temperatures have been reported not only in the United States but

[1]Deceased.

[2]Present address: College of Fisheries, University of Washington, Seattle, Washington 98105.

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from Canada (Colgrove and Wood 1966), Ireland (Anonymous 1965a), England, Wales, and Scotland (Anonymous 1965b, 1966).

Chondrococcus columnaris-infected coarsefishes in the Hanford stretch of the Columbia River during the summer have usually been negligible but minor outbreaks of the disease have occurred when river temperatures reach approximately 15 C (Fujihara 1967). During 1970, however, epidemic disease conditions were observed among coarsefishes at Hanford and in the Wenatchee section of the Columbia River, which contributed to higher than normal infection of sockeye salmon (*Oncorhynchus nerka*) and possibly other salmonid species during upstream migration (Fujihara 1971).

Although many factors, such as physical and nutritional state of the salmon, exposure to various toxicants, fish ladders, (Fujihara and Hungate 1971), gas-bubble, and other diseases are involved, temperature appears to be the major stress involved with the infection of fish to *C. columnaris* disease. Since the disease is usually associated with warmwater temperatures, we initiated studies to investigate the possible aggravation of the endemic disease by discharge into the Columbia River of heated effluent water from nuclear reactors of the U.S. Atomic Energy Commission. In this paper, we describe the results of laboratory investigations on the possible variation in the susceptibility of juveniles of chinook salmon and rainbow trout due to age, size, species, water temperature, and crowding.

Materials and Methods

The sources of exposure to the disease were *C. columnaris* present as natural contaminants in river water and a virulent strain supplied by Dr. E. J. Ordal of the University of Washington. This strain, arbitrarily designated as H #1, was injected intraperitoneally into a yearling rainbow trout. When the fish showed external symptoms of the disease after 2 weeks, scrapings were taken from a lesion in the caudal area and streaked on plates of solid growth medium. A single colony was isolated after 24 hr incubation at 25 C and transferred to liquid medium. At approximately 18 hr, during the log phase of growth, the culture was centrifuged and washed three times with physiological saline and the cells lyophilized in a 5% skim milk suspension. Each experiment, which required exposure of fish to known concentrations of virulent cells, utilized a freshly lyophilized H #1 culture. The organisms were grown with shaking at 200 rpm for 18 hr in liquid medium at 25 C and diluted to the required exposure concentration with physiological saline.

The liquid medium used for all experiments was composed of 0.4% Bacto tryptone and 0.04% Bacto-yeast extract at *p*H 7.3. One percent agar was added when a solid medium was required. The fish were exposed to concentrations ranging from approximately 1.25×10^5 to 1.5×10^6 *C. columnaris* organisms per liter of exposure water. Heavy clumping of organisms, which occurred during the log phase of growth, was removed by filtering the culture through loosely packed glass-wool.

Fish used as test animals were juvenile hatchery-reared rainbow trout and chinook salmon with no known history of exposure to the disease. For acute exposures, groups of fish were transferred to glass jars to which were added known concentrations of virulent organisms. The fish were exposed from 25 to 50 min at 10–22 C and returned to holding jars with clean flowing water at similar temperatures for observation up to 7 days.

Some Effects of Temperature, Age, and Size on Resistance to the Disease

Juveniles of rainbow trout and chinook salmon were exposed to *C. columnaris* at different stages of development from the early stages of growth through several months. Ten fish were exposed for 1 half-hour at 20 C to approximately 2.5×10^5 organisms per liter of sterilized river water and returned to holding jars with flowing dechlorinated water at a similar temperature for 7 days observation. The older and larger fish were less susceptible than the younger and smaller fish to *C. columnaris* (Fig. 1). When the younger and smaller fish were exposed to the organisms for 0.5 hr, less than 24 hr were required to kill 100% of the rainbow trout and 90% of the chinook salmon, whereas when several months old the fish survived the 7-day test period. This does not mean, however, that rainbow trout and chinook salmon of similar size and weight are equally resistant to the disease. At similar weights of approximately 1.25 g, the rainbow trout were resistant to the exposure conditions, whereas nine of the 10 chinook salmon were killed in less than 24 hr.

To distinguish between age and size as factors in resistance, two groups of 28 and 30 chinook salmon between 48 and 70 mm and between 85 and 105 mm in fork length, all 10 months old, were exposed to the pathogen. The two size groups had similar mortalities (81 and 83%) when exposed for 1 half-hour in water containing approximately 9×10^5 organisms per liter at 20 C. Median time to death was 28 hr for both groups of

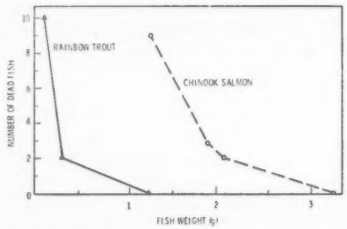

FIG. 1. Mortalities during 7 days of young rainbow trout and chinook salmon exposed for 1 half-hour at 20 C to approximately 2.5×10^5 *C. columnaris* per liter at weights of 0.12–1.2 g (rainbow trout) and 1.25–3.25 g (chinook salmon). Ten fish used at each of the selected weights.

fish. Although only one age-group of fish was tested, the findings suggest that size is probably not as important a factor as age in resistance among young chinook salmon.

Effects of Temperature and Crowding on Mortality

Sibling chinook salmon averaging 3.9 g that were held for 7 days in dechlorinated water at 10, 14, 18, 20, and 22 C were exposed for 25 and 50 min to approximately 2.5×10^5 and 1.25×10^5 *C. columnaris* per liter of sterile river water, respectively. These fish were held at the same temperatures in dechlorinated water until 100% mortality was observed. Although only three fish were used for each test condition, the limited data (Fig. 2) suggested a shorter survival time at the warmer temperatures. Doubling the exposure concentration and halving the exposure time had no substantial effect on mortality. The lethal effect at the lowest temperature (10 C) was noteworthy since *C. columnaris*-induced mortality of juveniles of the two species reared in Columbia River water is not observed during cool temperatures of late winter and early spring. Mortality of these 10 C fish was attributed to the high exposure concentrations used in the test, which could not occur under natural winter river conditions.

For seasonal studies on crowding and its relation to *C. columnaris* disease, juvenile chinook salmon were exposed to organisms entering the laboratory troughs from the river. The troughs were $1 \times 1 \times 10$ ft, and river water flow was adjusted to 4 gal per minute. Fish were held in lots of 50, 150, 450, and 900 from the first records of disease in early July, during an upward trend in river temperatures, through the end of October, when the temperatures were declining. Mortality was recorded and all dead fish examined for both exposure and infection. More than 10 surface isolates or one or more internal isolates from each fish were arbitrarily taken as an infection and isolation of 1 to 10 surface organisms as an exposure.

Crowding was an important factor in incidence of the disease. Although temperatures in all troughs increased from 17.5 to 21 C during the experiment, and the fish were exposed to *C. columnaris* disease, no infection or mortality was found in the 50-fish trough. Crowding hastened cross-infection and mortality in the more crowded troughs, the mortalities in the 150, 450, and 900-fish troughs being 1.3, 10.2, and 12.0 %, respectively. The average weight of the fish increased from 4.3 g initially, to 80.7, 86.2, 65.8, and 51.3 g for the 50-, 150-, 450-, and 900-fish troughs, respectively. The weight gains during the 4-month period in the 50-and 150-fish troughs of approximately 18-fold and 19-fold, respectively, as compared to a lower gain of about 11-fold in the 900 fish trough also show the beneficial results of holding fish in less crowded conditions.

Four replicates of 300 juvenile rainbow trout each were held in river water troughs from June for 21 weeks during the summer–fall *C. columnaris* season to examine the frequency distribution of deaths in each trough population. Dead fish were routinely checked for *C. columnaris* exposure and infection. Total mortality attributed to the disease (Fig. 3) during the first 4 weeks in the four troughs was 4.7, 6.7, 7.0, and 22%. Heaviest mortality occurred in each trough at temperatures ranging from 17.2 to 17.8 C, and not during peak temperatures in the late summer, which reached 20.6 C. Variation in mortality among troughs during the early part of the test may have been determined by the virulence of the dominant *C. columnaris* strain initially established in each trough. The 4.7, 6.7, and 7.0 % mortalities at 4 weeks in three of the troughs were probably caused by *C. columnaris* strains of similar virulence; the markedly higher mortalities (22%) in the other trough suggests that a strain of greater virulence was established.

The abrupt rise in mortality, followed by an equally abrupt decrease maintained for the rest of the season (Fig. 3), suggests that many of the trout may have developed a protective mechanism against the disease. The decreased mortality during the later period may also

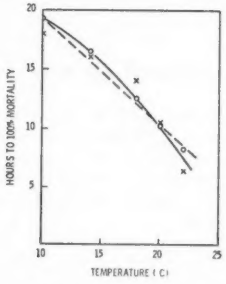

FIG. 2. Hours to 100% mortality of young chinook salmon exposed to approximately 2.5×10^5 and 1.25×10^5, respectively, of *C. columnaris* organisms per liter for 25 and 50 min, and held at 10, 14, 18, 20, and 22 C. Three fish averaging 3.93 g were used for each temperature and exposure concentration.

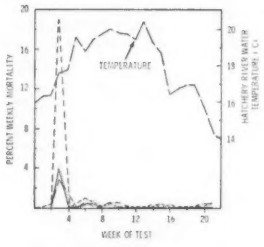

FIG. 3. *Chondrococcus columnaris* — associated mortalities in four replicate lots of 300 rainbow trout exposed to Columbia River hatchery water for 21 weeks. Mortalities of fish with more than 10 external isolates or one or more internal isolates were arbitrarily considered to be caused by *C. columnaris* disease. The fish averaged 2.4 g at the beginning and 63 g at the end of the test.

have been caused in part by increased natural resistance accompanying growth of the fish.

The effects of temperature on juvenile rainbow trout exposed to *C. columnaris* were investigated in hatchery river water at temperatures ranging from 17.7 to 21.7 C and with river water chilled 2.2 degrees C below and heated 2.2 degrees C above ambient temperatures (Table 1). Single troughs, each with 500 fish of the same age-group, were maintained at the three temperature conditions from July for 23 weeks. The disease was established in each of the troughs through exposure to organisms normally present in river water. Total mortality was divided into death from *C. columnaris* disease (infected fish, as defined previously) and death from unknown causes. Since all the fish were exposed to the pathogen, the cause of death may be speculative. An increase of 2.2 C above normal hatchery river temperatures greatly increased mortality rates from all causes, but the mortality differences between fish held at ambient and decreased temperatures were not appreciable. The cooler temperature, however, increased average weight by approximately 30% and generally reflects a more suitable environmental temperature for the fish.

Discussion

Many complex factors other than increased temperatures and crowding are involved in mortality of fish from *C. columnaris* disease. Some of these factors are probable immunity of previously exposed fish; differences in resistance to *C. columnaris* according to species, age, and condition of fish; differences in strain virulence of the *C. columnaris* organism; and interrelation with other fish diseases. As an example, the interaction of *C. columnaris* disease and gas-bubble disease may have a synergistic effect on mortality. The mortality of seaward-migrating salmon, as high as 90% for some species, has been directly attributed to supersaturation of river water with nitrogen or other gas from spillage of excess water over hydroelectric dam spillways, and warm river temperatures. Since *C. columnaris* disease is prevalent in the river during the same period, gas-bubble disease can result in a secondary *C. columnaris* infection, which may be the primary cause of mortality. The stresses induced on anadromous salmon by gas-bubble disease, and particularly the presence of ruptured gas-blisters that open "portals of entry," make these fish particularly susceptible to *C. columnaris* infection.

TABLE 1. Mortalities of single lots of 500 juvenile rainbow trout exposed to *C. columnaris* disease and average weights of the fish after 23 weeks at 3 temperature conditions.

	Mortality (%)			
Temp (*C*)	Total	Associated with *Columnaris* infection[a]	Not associated with *Columnaris* infection[b]	Avg Wt(*g*)
Ambient hatchery river water (17.7–21.7)	19.1	10.4	8.7	53.3
Ambient minus 2.2	13.7	6.2	7.5	76.9
Ambient plus 2.2	66.5	29.9	36.6	40.6

[a]More than 10 *C. columnaris* external isolates/fish or one or more *C. columnaris* internal isolates/fish.
[b]Ten or fewer *C. columnaris* external isolates/fish.

Chondrococcus columnaris infection of fishes is generally detected in the Columbia River in early July, when water temperatures reach approximately 15 C, whereas peak temperatures in the river during the summer months are between 20 and 21 C. When river temperature drops below 10 C following the summer season, *C. columnaris* generally cannot be isolated from hatchery salmonids or from native fish in the river. However, it seems reasonable to assume that certain fish may act as reservoir hosts capable of holding the pathogen during the winter months and reinfecting other fish the following spring.

Apparently juvenile rainbow trout and juvenile chinook salmon are susceptible in varying degrees to *C. columnaris* disease. Age, rather than size, per se, may be a major factor in their resistance to the pathogen.

Since crowding of the fish appears to play a major role in increasing the incidence of the disease, fish ladders, spawning channels, and warmwater slough areas that have relatively large populations of river fish may be loci of *C. columnaris* infection in river systems.

Acknowledgments

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A Biochemical and Cytological Study of the Longnose Sucker (*Catostomus catostomus*) and Large and Dwarf Forms of the White Sucker (*Catostomus commersoni*)

R. J. BEAMISH[1]

Department of Zoology
University of Toronto, Toronto, Ont.

AND H. TSUYUKI

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

BEAMISH, R. J., AND H. TSUYUKI. 1971. A biochemical and cytological study of the longnose sucker (*Catostomus catostomus*) and large and dwarf forms of the white sucker (*Catostomus commersoni*). J. Fish. Res. Bd. Canada 28: 1745–1748.

White (*Catostomus commersoni*) and longnose (*C. catostomus*) suckers possess diploid complements of 98 chromosomes, including metacentrics, submetacentrics, and acrocentrics. White sucker karyotypes differ consistently from longnose karyotypes by the presence of an additional four metacentrics. The karyotypes of the size and age at maturity of variants of white suckers were indistinguishable. Biochemically, longnose and white suckers are distinctive with respect to muscle myogens, hemoglobins, serum esterases, serum and muscle lactate dehydrogenases, and serum transferrins. The last group of proteins provides a clear genetic separation of the large-sized, late-maturing, and the dwarf, early-maturing white suckers. The former is polymorphic for serum transferrins.

BEAMISH, R. J., AND H. TSUYUKI. 1971. A biochemical and cytological study of the longnose sucker (*Catostomus catostomus*) and large and dwarf forms of the white sucker (*Catostomus commersoni*). J. Fish. Res. Bd. Canada 28: 1745–1748.

Le meunier noir (*Catostomus commersoni*) et le meunier rouge (*C. catostomus*) possèdent chacun un stock diploide de 98 chromosomes médiocentriques, submédiocentriques et acrocentriques. Le caryotype des meuniers noirs se distingue essentiellement de celui des meuniers rouges par la présence de quatre chromosomes médiocentriques supplémentaires. Il est impossible d'identifier le caryotype des meuniers noirs qui ont subi une mutation affectant la taille et l'âge à maturité. Biochimiquement, les deux espèces se distinguent par leurs myogènes musculaires, hémoglobines, estérases sériques, lacticodéshydrogénases sériques et musculaires et transferrines sériques. Ce dernier groupe de protéines permet de différencier génétiquement le grand meunier noir à maturité tardive de la forme naine à maturité hâtive. Le grand meunier noir est polymorphe quant aux transferrines sériques.

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DURING a study of factors affecting the age and size of the white sucker *Catostomus commersoni* at maturity it was desirable to determine if genetic differences existed between two allopatric populations. Two populations from lakes located in Killarney Provincial Park, Ontario, were studied. A rapid-growing large form existed in George Lake, whereas in Lumsden Lake, an extremely slow-growing and early-maturing form was found. For a complete description of the lakes and their sucker populations, see Beamish (MS 1970).

Dence (1948) was unable to find morphometric distinctions between sympatric rapid- and slow-growing forms of white suckers. Because in some investigations cytological studies have proven successful in the differentiation of polymorphic forms of a species (Setzer 1970; Thorneycroft 1966), this approach was used to investigate possible genetic differences between the two forms. Karyotypes were studied from the two forms of the white sucker as

[1]Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA.

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well as from the closely related longnose sucker, *Castostomus catostomus*.

A biochemical approach, used successfully in distinguishing, for example, sibling species of sea cucumbers *Thyonella gemmata* (Manwell and Ann Baker 1963) and several species of morphologically indistinguishable rockfishes (Tsuyuki et al. 1968), was also used. Genetically linked polymorphism is known to exist in the muscle myogens of *C. catostomus* (Tsuyuki et al. 1967). Other variations in the electrophoretic patterns of muscle myogens and plasma proteins in several species of catostomids have also been reported (Tsuyuki et al. 1967). Variation in the serum esterases of *Catostomus clarki* is probably controlled by a two-allele system at a single locus (Koehn and Rasmussen 1967). Polymorphisms in the hemoglobins of a number of species of catostomids have been described previously (Tsuyuki et al. 1967; Koehn 1969). The general topic of assessing genetically distinct populations of fishes by serological and biochemical techniques has been reviewed by de Ligny (1969).

Methods

White suckers were obtained from Lumsden Lake (slow-growing, early-maturing), George Lake (fast-growing), and the Don River in Toronto, Ont. (intermediate size). Longnose suckers were the offspring of fish taken from Lake Huron and reared from eggs at the Laboratory for Experimental Limnology, Maple, Ont. In general, small young fish were preferred for chromosome squashes.

Chromosome squashes were prepared using a modification of the technique of McPhail and Jones (1966). For a detailed description of the method see Beamish (MS 1970) or Beamish et al. (1971). For catostomids, an intramuscular injection of 0.1 cc of 0.1% colcemid for 4 hr produced suitable preparations. Only good quality squashes were scored, and counts were obtained by photographing squashes and counting chromosomes from the photograph with reference to the original squash.

The electrophoretic mobilities of various groups of proteins from longnose suckers, as well as the rapid- and slow-growing forms of white suckers, were compared. No proteins from the intermediate-size Don River white suckers were analyzed. The muscle extracts and blood proteins were prepared for starch-gel zone electrophoresis as described by Tsuyuki et al. (1962, 1965, and 1967). The method of preparing plasma transferrins for autoradiography by the method of Giblett et al. (1959) was described by Tsuyuki et al. (1969). Comparisons of the two white sucker variants and the longnose sucker were made using hemoglobins, serum esterases, muscle myogens, serum transferrins, and lactate dehydrogenase in muscle, heart, and liver. Extracts were prepared from fresh material and analyzed immediately, thus avoiding problems associated with storage.

Results

Karyotype Analysis

Counts made of 182 squashes obtained from 9 longnose suckers and 12 white suckers were divided into the following two categories: squashes in which there was no significant overlap of chromosomes were classified as "very good," and squashes in which chromosomes were well spread but chromosome overlap could cause errors in interpretation were termed "good." Only preparations that showed no obvious signs of chromosome loss were counted.

All white and longnose suckers had a diploid complement of 98 chromosomes. The frequency of chromosome numbers obtained for longnose suckers and Lumsden and George Lake suckers is presented in Fig. 1. Squashes from Lumsden Lake white suckers were obtained from older fish and, in general, were of poorer quality than those of George Lake origin. Squashes prepared from longnose suckers yielded preparations of better quality than either George or Lumsden Lake fish. The deviation from the value $2n = 98$ according to the quality of the preparation is evident from Fig. 1. Values greater than 98 appeared to be the result of chromatid separation or breaks during squashing, or both. Similarly, it was felt that counts lower than 98 resulted from chromosome loss during squashing. Counts of 97 were found for both male and female suckers. The inability to interpret chromosomes in some preparations was considered to be a prime reason of variation in chromosome number. In general, when squashes of the quality shown in Fig. 2 were counted, the resulting diploid number was consistently 98.

The complements of both species include metacentrics, submetacentrics, and acrocentrics. Fourteen karyotypes prepared from seven different George Lake white suckers were identical. Similarly, no differences were observed among the 13 karyotypes of Lumsden Lake white suckers or the karyotypes of 13 longnose suckers.

A comparison of white and longnose karyotypes (Fig. 3–6) shows that the longnose karyotype (Fig. 4) was readily distinguishable from that of the white sucker (Fig. 3, 5, and 6). The diploid complement of white suckers contains 12 metacentrics, whereas, the longnose diploid complement possesses eight metacentrics. No diagnostic chromosome differences could be found among the size variants of white suckers from George Lake, Lumsden Lake, or the Don River (Fig. 3, 5, and 6).

Biochemical

No diagnostic differences between Lumsden and George Lake white suckers were found for hemo-

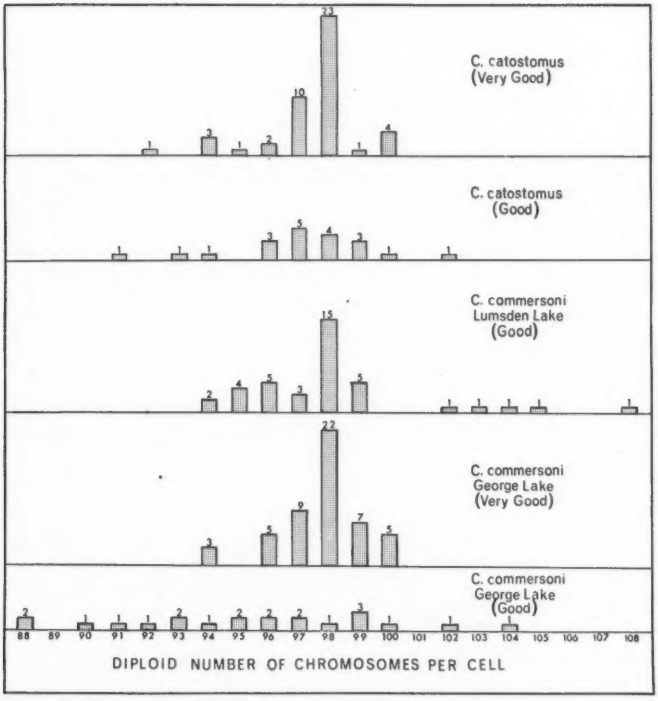

FIG. 1. Frequency distribution of chromosome counts of longnose and white suckers.

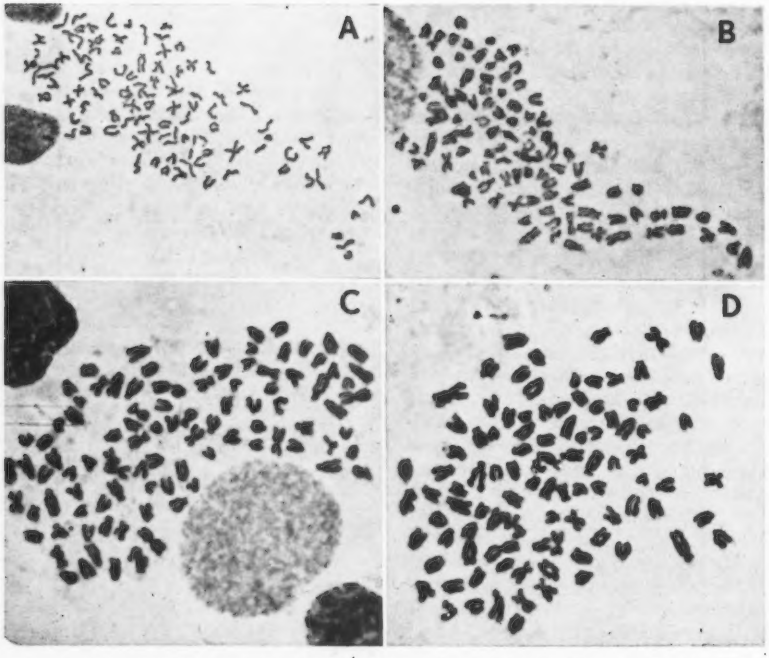

FIG. 2. Representative squashes from white and longnose suckers. A, George Lake white sucker; B, Lumsden Lake white sucker; C, Don River white sucker; D, longnose sucker.

FIG. 3. Karyotype of Lumsden Lake white sucker.

FIG. 4. Karyotype of longnose sucker.

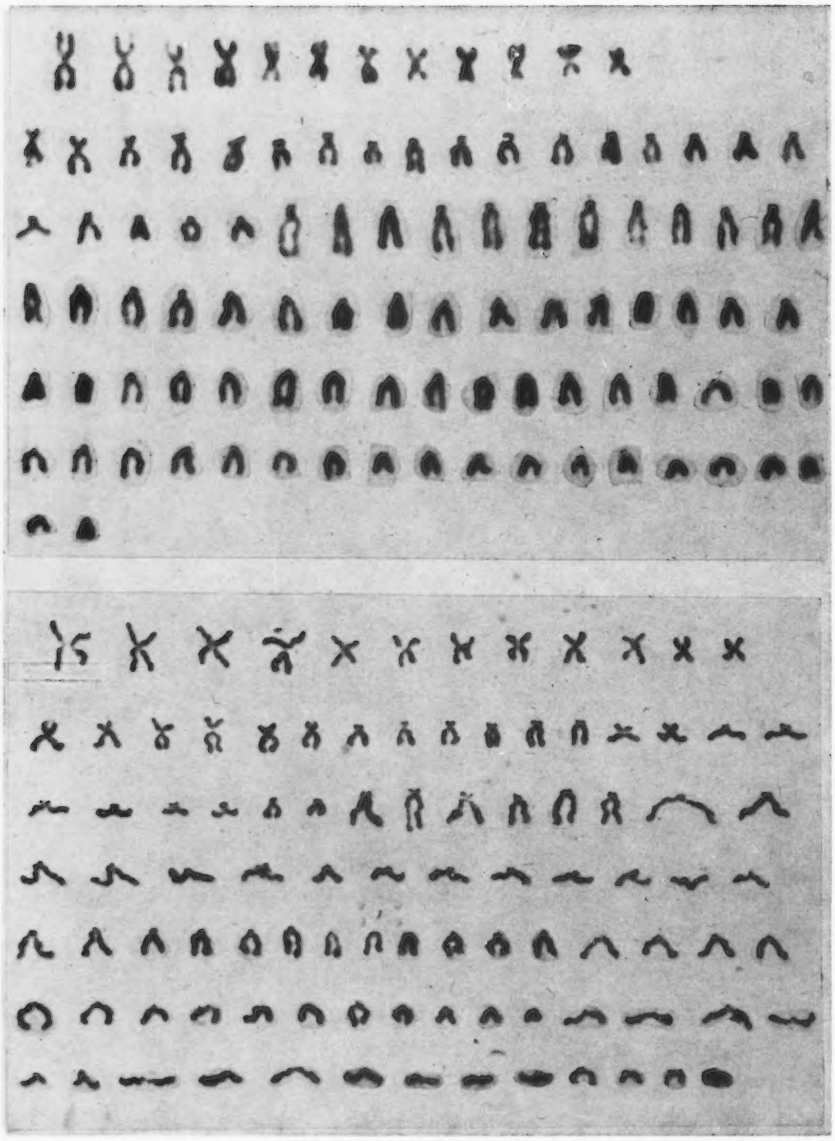

FIG. 5. Karyotype of Don River white sucker.

FIG. 6. Karyotype of George Lake white sucker.

globins, esterases, and muscle myogens. Although some differences between the George and Lumsden suckers were found in the lactate dehydrogenase systems in muscle, heart, and liver, these differences were not sufficiently distinct to allow a separation of the two forms on this basis alone. In the plasma transferrins, however, a diagnostically useful character was found. A diagram of the transferrin bands of the zymograms identified by autoradiography is shown in Fig. 7. The five Lumsden specimens (Fig. 7, no. 12–16) differed consistently from the George Lake suckers (Fig. 7, no. 7–11). The transferrins of the George Lake variant appear in polymorphic form, designated AA, AB, and BB. The George and the Lumsden forms do not appear to have any transferrins in common, and the electrophoretic mobilities of the transferrin molecules of the two forms differ widely.

The longnose sucker possesses transferrins of mobility somewhat similar to the Lumsden form (Fig. 7, no. 2–6), but the white and the longnose suckers are clearly distinguishable by their hemoglobins, muscle, and other protein systems (Tsuyuki et al. 1967).

Discussion

Cytologically, white suckers could readily be distinguished from longnose suckers by the presence of four additional metacentrics in the total complement of $2n = 98$ chromosomes. Change in the karyotype has resulted without a change in the chromosome number; hence, the Robertsonian phenomenon of centric fusion does not account for the observed karyotype dissimilarities between these two species. Pericentric inversions may be involved. No variations in chromosome number of morphology were observed for white suckers from different habitats.

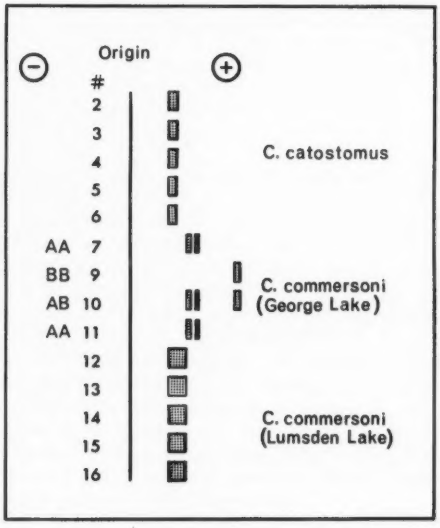

FIG. 7. Diagram of transferrin bands of the zymograms identified by autoradiography.

No diagnostic chromosome differences were observed in the karyotypes of white suckers representing different rates of growth and age at maturity. This does not necessarily mean that the karyotypes are similar, since the large number of acrocentric chromosomes of continuous gradation could obscure changes caused by translocations. The large number of chromosomes possessed by these species may be indicative that their ancestors may have at one time undergone a doubling of their chromosome complement (see Ohno et al. 1967 and 1968).

Cytological studies were inconclusive with respect to genetic differences between the various forms of white suckers. Although neither the effects of sialic acid and other factors influencing the electrophoretic mobility of serum transferrins nor the effect of oxidation levels of iron (Gaber and Aisen 1970) as possible causes of formation of double zoning in phenotype AA have been investigated at this time, a consistent separation of the George and Lumsden suckers was possible using this protein. In spite of the small sample, the Lumsden and George Lake suckers have to be regarded as genetically distinct forms with respect to this serum protein. The similarity of the other protein systems suggested that a close association did exist some time in the past.

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Effects of Starvation, Feeding, and Light Period on Circulus Formation on Scales of Young Sockeye Salmon (*Oncorhynchus nerka*)

H. T. BILTON AND G. L. ROBINS

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

BILTON, H. T., AND G. L. ROBINS. 1971. Effects of starvation, feeding, and light period on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 1749–1755.

Young sockeye salmon (*Oncorhynchus nerka*) survived a starvation period of 20 weeks with no change in their mean length and weight and with no record of this starvation period on their scales. Fish offered an ad libitum diet for more than 4 weeks in the 20-week period had significant increases in body and scale growth. The number of checks on their scales was correlated with the number of weeks fish were offered an ad libitum diet and not with the number of weeks they were starved. Thus, a significant increase in food induced the formation of a check. Light period appeared to have some influence on body and scale growth.

BILTON, H. T., AND G. L. ROBINS. 1971. Effects of starvation, feeding, and light period on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 1749–1755.

Des saumoneaux sockeye (*Oncorhynchus nerka*) ont survécu à une période de jeûne de 20 semaines sans que la croissance en longeur et en poids n'ait été affectée et sans que les écailles n'aient enrégistré de marques. Les poissons auxquels on a offert une diète ad libitum pendant plus de 4 des 20 semaines ont exhibé une augmentation sensible de la croissance du corps et des écailles. Le nombre de marques annulaires supplémentaires sur les écailles était fonction du nombre de semaines pendant lesquelles les poissons avaient libre accès à la nourriture plutôt que du nombre de semaines de jeûne. L'augmentation appréciable de la quantité de nourriture a donc causé la formation de marques supplémentaires sur l'écaille. La durée de l'éclairage a également semblé agir sur la croissance du corps et de l'écaille.

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FEEDING level influences circulus formation and the incidence of checks on scales of young sockeye salmon (*Oncorhynchus nerka*) (Bilton and Robins 1971a). Results indicated that the number and spacing of circuli and the scale radius were all correlated with the feeding level. There was a suggestion that check formation was associated with an increase in the amount of food. To extend our knowledge of the effect of environmental factors, this paper presents data on the effects of starvation, feeding, and light period on scale growth. As was stated earlier (Bilton and Robins 1971b), an understanding of the ways in which such factors influence circulus formation will provide a better basis for aging salmon from their scales.

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Materials and Methods

EXPERIMENTAL STOCK

The sockeye used in this study were reared at the Nanaimo Biological Station from eggs obtained from the lower Babine River (B.C.) in the autumn of 1968.

On November 25, 1969, 100 juvenile sockeye were selected without known bias from this donor stock and their fork lengths measured in millimeters, giving a mean and standard deviation of 88.99 ± 10.69 mm. On November 27, 340 fish whose lengths fell within 1 standard deviation of the above mean were selected for the experiment, and on November 28 were divided without known bias into 17 groups of 20 fish (Table 1).

Sixteen of these groups (A–P) were assigned randomly to tanks. The 17th group (Z) was measured for length, weight, and scale parameters, and killed. Data from this latter group served as the "starting point" against which subsequent growth data were compared.

Ration

To test the gross effects of ration, fish were offered either an ad libitum diet (commercial hatchery food) daily or were starved. The prolonged effects of each were examined, together with the effects of alternate periods of feeding and starving (Table 1).

Experimental Apparatus

As only the effects of ration and light period were to be examined, water temperature and water flow were held constant. Inflowing water was filtered and those tanks containing groups of fish that were reared in continuous fluorescent light or dark were covered with light-

Table 1. Experimental design: group designations and related periods of light and ad libitum daily feeding, or light and starvation.

Group	Nov. 28	Dec. 25/26	Jan. 22/23	Feb. 18/19	Apr. 16
A	Starved continuous light — 20 weeks				
M	Starved 12 hr light — 20 weeks				
O	Starved 12 hr light — 20 weeks				
G	Starved continuous dark — 20 weeks				
B	Fed daily continuous light — 20 weeks				
N	Fed daily 12 hr light — 20 weeks				
P	Fed daily 12 hr light — 20 weeks				
H	Fed daily continuous dark — 20 weeks				
All following groups reared under 12 hr light:					
I	Fed daily — 4 weeks		Starved — 8 weeks		Fed daily — 8 weeks
J	Fed daily — 4 weeks		Starved — 8 weeks		Fed daily — 8 weeks
K	Starved — 4 weeks		Fed daily — 8 weeks		Starved — 8 weeks
L	Starved — 4 weeks		Fed daily — 8 weeks		Starved — 8 weeks
C		Fed daily — 8 weeks		Starved — 4 weeks	Fed daily — 8 weeks
D		Fed daily — 8 weeks		Starved — 4 weeks	Fed daily — 8 weeks
E		Starved — 8 weeks		Fed daily — 4 weeks	Starved — 8 weeks
F		Starved — 8 weeks		Fed daily — 4 weeks	Starved — 8 weeks

proof boxes. Other groups were reared in 12 hr fluorescent light and 12 hr darkness. Light period was automatically controlled. Mean water temperature during the experiment was 4.95 ± 0.78 C. The 16 tanks were arranged in a room sealed from noise and light as described previously (Bilton and Robins 1971a).

Experimental Design

The experimental design for groups A–P is depicted in Table 1. All groups were sampled on April 16, except G, which was sampled on April 22. At this time, fish in groups B–F, H–L, N, and P were killed.

Fish in the remaining groups (A, M, G, and O) were returned to the apparatus. Their growth from this point (April 16) will be the subject of a separate paper.

Sampling and Scale Measurements

Fish were measured for length and weight and sampled for scales as indicated in Table 1, using similar methods to those described previously (Bilton and Robins 1971a). Mean values obtained were compared for statistical significance with either *t*-tests or a Duncan's Multiple Range test (Li 1964).

Results

Group Z (the starting point) — For group Z on November 27 mean number of circuli was 16.25 and mean scale radius (A–N) 70.45 mm (Table 2). Mean length was 88.80 mm and mean weight 6.59 g. A mean number of 2.10 checks was observed on their scales. The mean number of circuli to the first check was 5.50 and from the first check to the second 5.35 circuli.

Comparison between groups A–P on April 16 and group Z sampled on November 28 — Between groups starved (A, G, M, O) throughout the 20-week period and those that had been offered about 4 weeks ad libitum diet (E, F), there was no significant change in mean length, weight, and total number of circuli (Table 3). However, for the 10 groups (B–D, H–L, N, and P) that had been offered from 8 to 20 weeks ad libitum feeding, there were significant increases in mean length, weight, total number of circuli, and scale radius over those of group Z.

There was no significant change in the mean spacing of circuli in 12 of the 16 groups from that of group Z. The exceptions were groups A (starved throughout, reared in continuous light), B (fed daily throughout, reared in continuous light), G (starved throughout, reared in continuous dark), and N (fed daily throughout, reared in 12 hr light), in all of which the spacing increased significantly.

On April 16, scales from groups A–P had from two to five well-defined checks (example Fig. 1) with means ranging from 1.85 to 3.83 (Table 2). The overall mean number of circuli in the first check was 5.42 ± 2.26 and in the second check 5.65 ± 1.75. *t*-Tests showed that these means were not significantly different from those of group Z ($P > 0.5$ in both cases) at the beginning of the experiment.

There were significant increases ($P < 0.5$) in the mean number of checks on scales of all groups that had been offered an ad libitum diet for 8–20 weeks (groups B–D, H–L, and N, P). For groups that had been starved for 20 weeks (groups A, G, M, O), there was no significant change in the mean number of checks ($P > 0.05$). Of the two groups that had been offered an ad libitum diet for 4 weeks (groups E, F), group F had a significant increase in the mean number of checks ($P < 0.01$), but group E did not ($P > 0.05$).

The mean number of checks for each group was significantly and positively correlated ($r = 0.684$, $n = 16$, $P < 0.01$) with the number of weeks they had been offered a daily ad libitum diet.

Comparison within groups A–P on April 16 (Table 3) — Means of length, weight, and total circuli in groups either starved throughout (A, G, M, O) or offered an ad libitum diet daily for 4 weeks (E, F) were significantly smaller than those of groups offered an ad libitum diet daily for a total of 8–20 weeks. There were significant differences in the mean circulus spacing among many of the groups, but these appeared to be random differences that were not associated with the period of time they had been starved or offered an ad libitum diet.

There were highly significant positive correlations between the body and scale measurements at the 1% level, excluding circulus spacing, and the number of weeks groups were offered a daily ad libitum diet.

Correlation coefficients (r) are as follows ($n = 16$ in each instance): length of fish, +0.899; weight of fish, +0.949; total circuli, +0.893; circulus spacing, +0.091.

Comparison on April 16 within groups reared under different light periods, that had been either starved (A, G, M, O) or offered an ad libitum diet daily (B, H, N, P) November 28–April 16 (Table 3) group A (continuous light), G (continuous dark), M and O (12 hr light), all starved — No significant differences in mean length and weight were observed. There was no significant difference in total circuli among groups A, G, M or A, G, O, but group M had significantly more circuli than group O, even though both were reared in 12 hr light. In addition, there was no significant difference in circulus spacing among groups A,

TABLE 2. The mean length (mm), weight (g), and scale characters of fish in group Z on November 28 and groups A–P on April 16. CL = continuous light; CD = continuous dark; 12L = 12 hr light.

			Starved				Fed daily				12L							
			CL	12L		CD	CL	12L		CD	Alternated periods of feeding or starvation							
		Z	A	M	O	G	B	N	P	H	I	J	K	L	C	D	E	F
Length	$\bar{X}$	88.80	90.94	88.60	88.40	90.30	116.94	115.22	111.57	101.50	100.76	100.05	96.35	97.79	111.40	113.11	89.10	91.21
	SE	1.52	1.65	1.52	1.52	1.52	1.65	1.60	1.82	1.70	1.48	1.56	1.65	1.56	1.76	1.60	1.56	1.56
	N	20	17	20	20	20	17	18	14	16	21	19	17	19	15	18	19	19
Weight	$\bar{X}$	6.59	5.06	5.11	5.05	5.53	16.32	17.79	16.45	12.46	11.51	11.64	9.27	9.70	15.01	16.68	6.65	7.10
	SE	0.55	0.59	0.55	0.55	0.55	0.59	0.58	0.65	0.61	0.53	0.58	0.59	0.56	0.63	0.58	0.56	0.56
Total circuli	$\bar{X}$	16.25	17.53	17.80	16.00	16.65	21.76	21.38	22.85	20.62	20.14	20.05	19.59	19.95	21.47	21.44	17.68	17.47
	SE	0.52	0.56	0.52	0.52	0.52	0.56	0.54	0.62	0.58	0.50	0.53	0.56	0.53	0.60	0.54	0.53	0.53
Total scale radius (A)	$\bar{X}$	88.25	101.53	94.40	89.25	95.65	120.76	122.00	122.07	105.06	104.43	106.84	102.65	111.47	119.20	116.33	94.05	98.53
	SE	3.11	3.38	3.12	3.12	3.12	3.38	3.28	3.72	3.48	3.04	3.19	3.38	3.19	3.60	3.28	3.19	3.19
Scale radius (A–N)	$\bar{X}$	70.45	84.53	77.15	73.15	78.95	104.29	105.22	106.00	89.06	86.95	89.84	84.88	93.05	100.93	98.50	77.74	80.16
	SE	2.90	3.14	2.90	2.90	2.90	3.15	3.06	3.47	3.25	2.83	2.98	3.15	2.98	3.35	3.06	2.98	2.98
Circuli spacing (A–N/C–1)	$\bar{X}$	4.63	5.12	4.58	4.87	5.07	5.02	5.16	4.84	4.53	4.55	4.72	4.59	4.92	4.95	4.81	4.67	4.86
	SE	0.11	0.12	0.11	0.11	0.11	0.12	0.12	0.13	0.13	0.11	0.11	0.12	0.11	.13	0.12	0.11	0.11
No. checks	$\bar{X}$	2.10	2.00	2.25	1.85	2.05	2.70	2.83	3.14	3.20	3.00	3.15	3.53	3.05	3.13	3.83	2.21	2.47
	SE	0.07	0.15	0.17	0.13	0.11	0.11	0.12	0.14	0.17	0.12	0.20	0.15	0.09	0.21	0.17	0.14	0.14
No. circuli, 1st check	$\bar{X}$	5.50	5.88	6.15	5.95	5.45	5.47	5.94	6.21	4.80	5.52	4.74	4.35	4.95	6.00	5.11	5.16	5.10
	SE	0.42	0.64	0.60	0.51	0.56	0.48	0.67	0.58	0.54	0.44	0.50	0.29	0.38	0.79	0.43	0.32	5.10
	N	20	17	20	20	20	17	18	14	15	21	19	17	19	15	18	19	19
No. circuli, 2nd check	$\bar{X}$	5.35	6.00	5.12	5.66	6.00	6.47	6.05	5.50	6.27	5.71	5.05	5.12	6.05	5.27	5.33	5.89	5.00
	SE	0.28	0.48	0.39	0.41	0.48	0.45	0.36	0.54	0.45	0.32	0.39	0.40	0.45	0.42	0.39	0.47	0.37
	N	20	14	16	15	18	17	18	14	15	21	18	17	19	15	18	18	19
No. circuli, 3rd check	$\bar{X}$	3.00	4.50	4.33	4.50	3.50	6.66	6.14	6.38	5.08	6.17	5.13	4.37	5.50	5.63	4.82	4.25	4.14
	SE	–	–	0.33	–	–	0.53	0.33	0.57	0.51	0.44	0.40	0.27	0.33	0.62	0.34	0.25	0.55
	N	2	3	9	2	3	12	13	13	18	18	15	16	18	11	17	4	7
No. circuli, 4th check	$\bar{X}$	–	–	–	–	–	–	7.00	6.33	4.40	6.33	5.87	5.20	5.00	6.50	4.78	6.00	3.00
	SE	–	–	–	–	–	–	–	0.88	0.60	0.88	0.67	0.33	–	0.72	0.78	–	–
	N	–	–	–	–	–	–	1	3	5	3	8	10	2	6	14	1	1
No. circuli, 5th check	$\bar{X}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.00	–	–
	SE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	N	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–

TABLE 3. Duncan's Multiple Range test comparing the means of length, weight, and scale characters of group Z on November 28 with groups A–P on April 16. Means not underscored by a common line differ significantly at the 5% level.

	Groups																
Length (*mm*)	O 88.40	M 88.60	Z 88.80	E 89.10	G 90.30	A 90.94	F 91.21	K 96.35	L 97.79	J 100.05	I 100.76	H 101.50	C 111.40	P 111.57	D 113.11	N 115.22	B 116.94
Weight (*g*)	O 5.05	A 5.06	M 5.11	G 5.53	Z 6.59	E 6.65	F 7.10	K 9.27	L 9.70	I 11.51	J 11.64	H 12.46	C 15.01	B 16.32	P 16.45	D 16.68	N 17.79
Total circuli (C)	O 16.00	Z 16.25	G 16.65	F 17.47	A 17.53	E 17.68	M 17.80	K 19.59	L 19.95	J 20.05	I 20.14	H 20.62	N 21.38	D 21.44	C 21.47	B 21.76	P 22.85
Nucleus radius (N)	H 16.00	P 16.07	O 16.10	E 16.31	B 16.47	N 16.67	G 16.70	A 17.00	J 17.00	I 17.09	M 17.25	K 17.76	Z 17.80	D 17.83	C 18.26	F 18.37	L 18.42
Total scale radius (A)	Z 88.25	O 89.25	E 94.05	M 94.40	G 95.65	F 98.53	A 101.53	K 102.65	I 104.43	H 105.06	J 106.84	L 111.47	D 116.33	C 119.20	B 120.76	N 122.00	P 122.07
Scale radius (A–N)	Z 70.45	O 73.15	M 77.15	E 77.74	G 78.95	F 80.16	A 84.53	K 84.88	I 86.95	H 89.06	J 89.84	L 93.05	D 98.50	C 100.93	B 104.29	N 105.22	P 106.00
Circulus spacing $\frac{A–N}{C–I}$	H 4.53	I 4.55	M 4.58	K 4.59	Z 4.63	E 4.67	J 4.72	D 4.81	P 4.84	F 4.86	O 4.87	L 4.92	C 4.95	B 5.02	G 5.07	A 5.12	N 5.16

G, O or G, M, O, but group A reared in continuous light had a significantly larger mean circulus spacing than group M reared in 12 hr light. There was also no significant difference in the scale radius among groups A, G, M or G, M, O, but group A reared in continuous light had a significantly greater mean scale radius than group O reared in 12 hr light.

Groups B (continuous light), H (continuous dark), N and P (12 hr light), all offered ad libitum diet — There was no significant difference in the mean length of fish between group B reared in continuous light, group N reared in 12 hr light, or between group N and group P both reared in 12 hr light. However, group B reared in continuous light was significantly larger than group P reared in 12 hr light; and groups B, N, P were all significantly larger than group H reared in continuous darkness.

The mean weights of fish in groups B, N, P did not differ significantly, but they had significantly greater mean weights than group H reared in continuous darkness. The mean total number of circuli among groups B, N, P or B, H, N did not differ significantly, but group P reared in 12 hr light had significantly more circuli than group H reared in continuous darkness.

There were no significant differences in the mean scale radius (A–N) or in the mean circulus spacing among groups B, N, P. However, the mean scale radius and mean circulus spacing of these groups were significantly greater than those of group H reared in continuous darkness.

Comparison on April 16 of the incidence of checks within groups reared under different light periods, that had been either offered a daily ad libitum diet (B, H, N, P) or starved throughout the period November 28–April 16 (A, G, M, O) — Group B, reared in continuous light, had a significantly lower mean number of checks (2.70) than either group H, reared in continuous darkness ($P < 0.05$), or groups N and P combined, reared in 12 hr light ($P < 0.05$).

Group H, reared in continuous darkness, did not differ significantly in the mean number of checks (3.20) from groups N and P combined, reared in 12 hr light ($P > 0.05$).

The incidence of checks for these groups (A, M, O, G) were not significantly different ($P > 0.05$) regardless of light period.

Discussion

Young sockeye that had been starved for a period of 20 weeks (November 28–April 16) while at a low temperature under laboratory conditions showed no change in their mean length or weight or in the total number of circuli on their scales. If the history of the fish had been unknown, it would have been impossible to ascertain from their scales that a period of starvation had occurred. Thus, in effect, 20 weeks of the fish's life were not recorded on the scales. During this time, only one starved fish out of 79 died. Those fish that had been offered an ad libitum diet for more than 4 weeks had significant increases in body and scale measurements.

It had been generally accepted that checks form during periods of low abundance of food. This may not be the case. The present results indicated that *the number of checks were correlated with the number of weeks fish were offered a daily ad libitum diet and not with the periods of starvation.* These results are contrary to the conclusions of Major and Craddock (1962), who concluded that starvation induced the formation of a check on the scales of young sockeye. However, as their fish were starved and then fed, and the scales examined for the presence of a check after a period of feeding, it is possible the check that they observed had not formed during the starvation period but during the period of feeding.

It is likely that rate of circulus formation is a function of a number of physical and nonphysical factors (e.g., time, temperature, food, light, maternal (Bilton MS 1970) and inherent factors). If these factors were held constant, circuli might form at a constant rate at a constant distance from each other. However, if one factor was changed, e.g., food intake was increased, then the rate of circulus formation would increase, resulting in more closely spaced circuli and the formation of a check.

Light period appeared to have some influence on body growth and circulus formation. Although this warrants further study, some speculation may be justified at this time. For the starved fish, continuous light and continuous darkness appeared to influence the scale radius and circulus spacing as both increased during the 20-week period, even though the body size and number of circuli did not differ significantly from group Z at the beginning, or the other starved groups. It is not possible to account for this change, based on present data. The fact that there were increases in scale radius and circulus spacing that were not accompanied by increases in body size and number of circuli is contradictory. Outside of a sampling error, a possible explanation is that scale growth was out of phase with body growth. For fish offered a daily ad libitum diet, those reared either in continuous or 12-hr light increased more in body size and scale growth than those reared in continuous darkness. Thus, it would seem that lack of light reduced body and scale growth of the fish. On the other hand, fish reared in continuous light had a lower mean

FIG. 1. Scale of a sockeye from group P sampled on April 16. Checks are indicated by the arrows.

number of checks on their scales than those reared either in 12 hr light or in continuous darkness. Again, an explanation cannot be given.

No circuli were formed during the period of starvation. This was not as expected and is at variance with the results of Gray and Setna (1931) and Bhatia (1931), who found circuli formed more closely together during periods of starvation. In the present paper, results indicate the opposite; a significant increase in food induced a check.

Acknowledgments

The authors extend their thanks to Drs G. R. Bell, J. H. Mundie, R. R. Parker, and F. H. C. Taylor, and to Mr. S J. Westrheim for their helpful criticisms of the manuscript, and to Mr D. W. Jenkinson and Mrs M. M. Aarts for their technical assistance in preparing the data.

BHATIA, D. 1931. On the production of annual zones in the scales of the rainbow trout (*Salmo irideus*). J. Exp. Zool. 59: 45–49.

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1971b. Response of young sockeye salmon (*Oncorhynchus nerka*) to prolonged periods of starvation. J. Fish. Res. Bd. Canada 28: 1757–1761.

GRAY, J., AND S. B. SETNA. 1931. The growth of fish. IV. The effect of food supply on the scales of *Salmo irideus*. J. Exp. Biol. 8: 55–62.

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Response of Young Sockeye Salmon (*Oncorhynchus nerka*) to Prolonged Periods of Starvation

H. T. BILTON AND G. L. ROBINS

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

BILTON, H. T., AND G. L. ROBINS. 1971. Response of young sockeye salmon (*Oncorhynchus nerka*) to prolonged periods of starvation. J. Fish. Res. Bd. Canada 28: 1757–1761.

Young sockeye were held without food for periods of 20 and 30.5 weeks with few mortalities. As was reported in previous experiments, no record of the periods of starvation appeared on the scale, nor did checks form under these conditions. Those fish offered food after 20 weeks of starvation responded immediately and showed significant increases in body and scale growth at the end of 10 weeks feeding. Fish offered food after 30.5 weeks of starvation also responded immediately, but when the experiment ended after 5 weeks of feeding no significant increases in body and scale growth were apparent, indicating insufficient recovery time. A significant increase in the incidence of checks was associated with subsequent feeding. Light period did have some influence on body and scale growth.

BILTON, H. T., AND G. L. ROBINS. 1971. Response of young sockeye salmon (*Oncorhynchus nerka*) to prolonged periods of starvation.. J. Fish. Res. Bd. Canada 28: 1757–1761.

Des saumoneaux sockeye furent soumis à des jeûnes de 20 et 30.5 semaines sans qu'il en résulte de mortalité appréciable. Comme nous l'avons mentionné à la suite d'expériences antérieures, aucune trace de jeûne n'a apparu sur l'écaille. Après 20 semaines de jeûne, les poissons auxquels nous avons offert de la nourriture mangèrent immédiatement et, après 10 semaines, accusèrent une augmentation sensible de la croissance du corps et des écailles. Les poissons soumis à un jeûne de 30.5 semaines se mirent aussi à manger immédiatement. Toutefois, quand l'expérience s'est terminée après 5 semaines d'alimentation, le corps et les écailles ne manifestaient aucune croissance, signe d'une période de rétablissement trop courte. Par la suite, l'alimentation a produit une augmentation significative des marques annulaires supplémentaires de l'écaille. La durée d'illumination eut une certaine influence sur la croissance du corps et de l'écaille.

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PREVIOUS workers have suggested that starvation induces the formation of closely spaced circuli on the scales (Gray and Setna 1931; Bhatia 1931; Major and Craddock 1962). The latter have used this phenomenon as a means of marking individual groups of fish. However, Bilton and Robins (1971b) have evidence suggesting that checks are associated not with starvation but with subsequent feeding.

This report is concerned with the effects of prolonged periods of starvation on the growth of young sockeye, their response to food after periods of starvation, and the subsequent growth of the fish while reared under three light conditions. This study complements a larger experiment to determine the effects of starvation, feeding, and light period on the scale growth of young sockeye salmon (Bilton and Robins 1971b).

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Materials and Methods

EXPERIMENTAL STOCK

The sockeye used in this study were reared at the Nanaimo Biological Station from eggs obtained from the Lower Babine River, B.C., in the fall of 1968.

SELECTION OF EXPERIMENTAL GROUPS

The selection of fish for the experiment is described in Bilton and Robins (1971b).

On November 28 the 100 experimental fish were randomly divided into five groups of 20 fish. Four of these groups (A, G, M, O) were assigned without known bias to tanks and the fifth group (Z) was sampled for length, weight, and scales. The data so obtained from the latter group served as the starting point against which subsequent growth data were compared.

RATION

Following prolonged periods of starvation (Table 1) fish were fed ad libitum daily commercial diet in pellet form.

TABLE 1. Experimental groups and design of the experiment. Continuous light is indicated CL, continuous dark CD, 12 hr light by 12, ad libitum feeding by X, and starvation by 0.

Period one						Period two						Period three					
No. fish	Period	Food	Light	Sampled	Mortality	Divided into sub-groups	No. fish	Period	Food	Light	Sampled	Mortality	No. fish	Period	Food	Light	Sampled	Mortality
Group A																		
20	Nov. 28–Apr. 16	0	CL	Apr. 16	1	AA	9	Apr. 16–May 21	0	CL	May 21	6	–	–	–	–	–	–
						AC	5	" 16–June 30	X	CL	June 30	0	–	–	–	–	–	–
						AE	5	" 16– " 30	X	CL	"	1	–	–	–	–	–	–
Group G																		
20	Nov. 28–Apr. 22	0	CD	Apr. 22	0	GG	10	" 22– " 30	0	CD	"	0	9[e]	July 1–Aug. 5	X	CD	Aug. 5	5
						GB	5	" 22– " 30	0	CD	"	0	4[e]	" "	X	CD	"	1
						GH	5	" 22– " 30	0	CD	"	1	4	" "	X	CD	"	2
Group M																		
20	Nov. 28–Apr. 16	0	12	Apr. 16	0	MM	10	" 16– " 30	0	12	"	1	–	–	–	–	–	–
						MI	5	" 16– " 29	X	12	June 29	0	–	–	–	–	–	–
						MK	5	" 16– " 29	X	12	" 29	0	–	–	–	–	–	–
Group O																		
20	Nov. 28–Apr. 16	0	12	Apr. 16	0	O	15[a]	" 16– " 22	0	12	" 22[b]	0	13[d]	June 23–Aug. 5	X	12	Aug. 5	5

[a]5 fish removed from population 0 on April 16 immediately after sampling, leaving 15 fish.
[b]2 fish sampled on this date. Both removed from population and preserved, hence only 13 fish beginning period 3.
[e]1 fish died on day of sampling.
[d]2 fish died on day of sampling.

Experimental Apparatus

The apparatus was essentially the same as used in a previous experiment (Bilton and Robins 1971a). Temperatures did not remain constant for the duration of the experiment and were as follows:

	Mean temp *C* (SD)
Nov. 28–April 16	4.95(0.78)
April 16–May 21	6.52(0.34)
April 16–June 22	7.89(1.87)
April 16–June 30	8.63(2.08)
April 16–Aug. 5	10.13(2.67)
June 30–Aug. 5	12.84(0.80)

Experimental Design

The experimental design for groups A, G, M, and O is shown in Table 1. With the exception of group G which was sampled on April 22, all fish were sampled on April 16. Immediately after sampling, groups A, G, and M were divided into subgroups (five individuals to each of the subgroups AC, AE, GB, GH, MI, and MK; ten individuals to each of the subgroups AA, GG, and MM). The first letter of the subgroup indicates the group origin and the second letter indicates the tank to which it was assigned. Group O was not subdivided.

Sampling, Scale Measurements, and Statistical Tests

Fish were measured for length, weight, and sampled for scales as indicated in Table 1, and data analysed as described previously (Bilton and Robins 1971a, b).

Results

A general summary of the results is given in Table 2. However, the detailed analyses and data have been omitted for the sake of brevity. This information is unpublished but can be obtained on request (Bilton and Robins MS 1971).

For the period November 28 to April 16 (20 weeks) groups A, G, M, and O were starved. The results for this period are discussed in Bilton and Robins (1971b). The results of the current experiment, separated by treatment (Table 2), are given in the following sections.

Comparison of subgroups (AC, AE, MI, MK) continuously starved from November 28 to April 16 (20 weeks), then fed from April 16 to June 30 (10.5 weeks) — From April 16 to June 30 mean length, weight, number of circuli, and scale radius

Table 2. Summary of comparison of changes in body and scale growth in groups on April 16 with group Z and in subgroups subjected to various periods of starvation and feeding. Significant increases and decreases at 5% level indicated by + and – respectively. No difference indicated by *.

	Within subgroups						Between subgroups	
Subgroup	Length	Weight	Total circuli	Scale radius (A–N)	Circulus spacing	Number checks	Weight[a]	Treatment
		June 30 vs. April 16					*June 30*	
AA	*	*			No scales obtained			
GB	*	–	*	*	*	*	*	Starved Nov. 28–June 30
GG	*	–	*	*	–	*	+0	Sampled April 16 and
GH	*	–	*	*	*	*	*	June 30
MM	*	–	*	*	*	*	*	
O	*	*	*	*	*	*	–GG[b]	
AC	+	+	+	+	*	*	*	Starved Nov. 28–Apr. 16,
AE	+	+	+	+	*	+	*	then fed to June 30
MI	+	+	+	+	+	*	+MK	Sampled April 16 and
MK	+	+	+	+	+	+	–MI	June 30
		Aug. 5 vs. June 30					*Aug. 5*	
GB	*	+	*	*	*	*	*	Starved Nov. 28–June 30,
GG	*	+	*	*	*	*	+0	then fed to Aug. 5
GH	*	*	*	*	*	*	*	Sampled June 30 and
O	+	*	*	*	*	–	–GG	Aug. 5

[a]Length, total circuli, scale radius, circulus spacing, and checks did not differ significantly between subgroups.
[b]Indicates GG was significantly less than 0.

of all subgroups increased significantly. There were significant increases in mean circulus spacing of subgroups MI and MK, both reared in 12 hr light, but not in AC and AE, both reared in continuous light. There was a significant increase in the mean number of checks in subgroups AE, reared in continuous light, and MK, reared in 12 hr light, but not in AC, reared in continuous light, and MI, reared in 12 hr light.

On June 30 there were no significant differences in mean length and weight of fish and scale characters between subgroups except in subgroups MI and MK, both reared in 12 hr light, where there was a significant difference in mean weight.

There was one mortality, which occurred in subgroup AE reared in continuous light.

Comparison of subgroups (GB, GG, GH, and group O) continuously starved from November 28 to June 30 (30.5 weeks), then fed from June 30 to August 5 (5 weeks) — From June 30 to August 5 mean length of subgroups did not change significantly, but in group O, reared in 12 hr light, there was a significant increase. The mean weight of subgroup GH, reared in continuous darkness, and group O, reared in 12 hr light, did not change significantly, but there was a significant increase in mean weight in subgroups GB and GG, both reared in continuous darkness. There were no significant differences in mean scale characters except in group O, reared in 12 hr light, where there was a significant decrease in the mean number of checks.

On August 5 there were no significant differences in mean length between subgroups. However, there was a significant difference in mean weight between subgroup GG, reared in continuous darkness, and group O, reared in 12 hr light. There were no significant differences in the mean scale characters between the subgroups and group O.

There were 13 mortalities of which 8 occurred in subgroups GB, GG, and GH, all reared in continuous darkness, and the remaining 5 in group O, reared in 12 hr light.

Comparison of subgroups (GB, GG, GH, MM, and group O) continuously starved from November 28 to June 30 (30.5 weeks) and subgroup AA to May 21 (25 weeks) — From April 16 to June 30 the mean length of all subgroups did not change significantly, but there were significant decreases in mean weight of all subgroups except AA (sampled on May 21) and group O.

There were no significant differences in means of scale characters except in subgroup GG, reared in continuous darkness, where there was a significant decrease in circulus spacing.

On June 30 there were no significant differences in mean length among subgroups, but there was a mean weight difference between subgroup GG, reared in continuous darkness, and group O, reared in 12 hr light. There were no significant differences in mean scale characters among the subgroups or group O.

There were 8 mortalities of which 6 occurred in subgroup AA reared in continuous light; the remaining 2 in GH reared in continuous darkness, and MM reared in 12 hr light.

Discussion

Young sockeye survived without food while at a low temperature under laboratory conditions for a period of 20 weeks without suffering large mortalities (1 out of 79 fish). During this time there was no change in their mean length or weight or in the total number of circuli on their scales, i.e., there was no record of this period on the scale (Bilton and Robins 1971b).

A number of fish were capable of enduring a longer period of starvation (30.5 weeks) but with increased mortalities (8 out of 54 fish). The mean number of checks and number of circuli on their scales remained unchanged. Thus, again, for an even longer period of starvation there was no record on the scale.

After both periods of starvation (20 and 30.5 weeks) fish responded almost immediately to the presence of food. Those which had been starved for 20 weeks and allowed to feed for 10.5 weeks all recovered and showed significant increases in body and scale growth. Thus, even after 20 weeks of starvation fish were capable of ingesting and utilizing food for growth. In two subgroups the mean number of checks increased significantly due to the increased availability of food. This supports our earlier findings (Bilton and Robins 1971a, b).

Those which had been starved for 30.5 weeks and allowed to feed for only 5 weeks did not show the increases in length, weight, or scale characters as did the 20-week starved, 10.5-week fed fish. It would appear therefore that sufficient recovery time had not elapsed when the experiment was terminated. This apparent relationship between starvation time and recovery time was also observed by Major and Craddock (1962). *It is interesting to note that again neither circuli nor checks formed during a period of little or no increase in body size.*

Light appeared to influence scale and body growth: in those groups starved for 20 weeks then fed for 10.5 weeks, the circulus spacing of those reared under 12 hr light had increased significantly but not in those reared in continuous light; at the end of 30.5 weeks starvation those reared

in continuous darkness had significant losses in weight as did one group reared in 12 hr light; those on continuous light and the one group on 12 hr light did not lose weight.

The fact that young sockeye can withstand long periods of starvation under laboratory conditions and are then capable of ingesting and utilizing food resulting in growth, is pertinent to the management of the species. Further, the fact that no record of the starvation period was evident on the scale must also be an important consideration when aging this species from its scales.

BHATIA, D. 1931. On the production of annual zones in the scales of the rainbow trout (*Salmo irideus*). J. Exp. Zool. 59: 45–49.

BILTON, H. T., AND G. L. ROBINS. 1971a. Effects of feeding level on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 861–868.

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Eutrophication of Lake 227, Experimental Lakes Area, Northwestern Ontario, by Addition of Phosphate and Nitrate

D. W. SCHINDLER, F. A. J. ARMSTRONG, S. K. HOLMGREN, AND G. J. BRUNSKILL

Fisheries Research Board of Canada
Freshwater Institute, Winnipeg, Man.

SCHINDLER, D. W., F. A. J. ARMSTRONG, S. K. HOLMGREN, AND G. J. BRUNSKILL. 1971. Eutrophication of lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate. J. Fish. Res. Bd. Canada 28: 1763–1782.

Addition of 0.34 g P as Na_2HPO_4 and 5.04 g N as $NaNO_3$ per square meter to a small unproductive Canadian Shield lake over a period of 17 weeks caused a severalfold increase in phytoplankton standing crop, and a change in dominant species from Chrysophyceae to Chlorophyta. Reactive phosphate concentrations remained at undetectable levels in the epilimnion after fertilization, and inorganic nitrogen concentrations remained low. Most of the added phosphorus and nitrogen was rapidly taken up by phytoplankton and sedimented with the seston.

Although concentrations of total CO_2 decreased to less than 20 μmoles/liter and *p*H values increased to greater than 9 in late summer, a high standing crop of phytoplankton was maintained. No marked increase in the rate of phytoplankton production was noticed. Experiments in polyethylene containers suspended in the lake during this period of low total CO_2 indicated that carbon was not limiting to algal production, except possibly at algal standing crops in excess of 100 μg/liter chlorophyll *a*, and that phosphorus was the primary limiting nutrient.

Sodium added with the nutrients remained in the epilimnion until fall overturn, indicating that very little of it was utilized by phytoplankton.

SCHINDLER, D. W., F. A. J. ARMSTRONG, S. K. HOLMGREN, AND G. J. BRUNSKILL. 1971. Eutrophication of lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate. J. Fish. Res. Bd. Canada 28: 1763–1782.

L'addition de 0.34 g de P sous forme de Na_2HPO_4 et de 5.04 g de N sous forme de $NaNO_3$ par m^2 à un petit lac non productif du bouclier canadien a eu pour effet d'augmenter considérablement la récolte permanente de phytoplancton et de remplacer les chrysophycés par les chlorophytes comme espèces dominantes. Après fertilisation, la teneur en phosphate réactif de l'épilimnion s'est maintenue à un niveau indécelable et celle de N inorganique est demeurée faible. La plus grande partie de l'apport de P et N a été rapidement absorbée par le phytoplancton et s'est déposée avec le seston sous forme de sédiment.

En dépit du fait que la teneur en CO_2 total ait baissé à moins de 20 μmoles/liter et que le *p*H soit monté à plus de 9 en fin d'été et malgré l'absence d'accroissement notable des taux de production du phytoplancton, la récolte permanente s'est maintenue à un niveau élevé. Des essais avec sacs de polyéthylène suspendus dans le lac durant la période de faible CO_2 total indiquent que le C n'est pas un facteur limitatif de production d'algues, sauf peut-être dans le cas d'une récolte permanente dépassant 100 μg/liter de chlorophylle *a*. D'après ces mêmes expériences, le P est le facteur limitatif primaire.

Le Na ajouté aux substances nutritives est demeuré dans l'épilimnion jusqu'au renversement automnal, signe d'une très faible utilisation par le phytoplancton.

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THIS is the first report on a program designed by Freshwater Institute staff to test the effect of known doses of nutrients upon natural whole-lake systems that have previously been uninfluenced by human activity. Lakes to be used for this purpose are located in the Experimental Lakes Area (ELA), northwestern Ontario (see J. Fish. Res. Bd. Canada 28(2), 1971).

The purpose of this paper is to describe the 1st year's results of a long-term program designed to test the effect of enriching a small, culturally undisturbed lake with low continuous doses of phosphorus and nitrogen, and to discuss possible applications of our findings to larger, economically more im-

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portant water bodies, such as the St. Lawrence Great Lakes.

Fertilization of lakes or ponds, usually to increase fish production, is a common procedure (see, for example, the reviews by Maciolek 1954; Mortimer and Hickling 1954; Winberg and Lyaknovich 1965). Most fertilization programs have included the addition of nitrogen and phosphorus, sometimes in combination with lime, organic fertilizers, or potassium salts. Most have recorded striking increases in algal abundance. Of particular relevance are the studies of fertilization in Canadian Shield lakes carried out by Langford (1950) and Smith (1948, 1955, 1961, 1968, 1969). The results of studies of phosphorus movement in small lakes by radiotracer techniques are also of interest (Hutchinson and Bowen 1947, 1950; Coffin et al. 1949; Hayes and Coffin 1951; Hayes and Phillips 1958; Rigler 1956). The four last-named studies were also carried out in Canadian Shield lakes. The relevance of the above studies to our work will be discussed below.

Description of the Lake

Lake 227 lies approximately 5 miles northeast of the ELA camp, at 49°42′N and 93°42′W. The location is shown in Cleugh and Hauser (1971). The lake is 5.0 hectares in area, roughly circular in outline, with a maximum depth of 10.0 m in the center of the lake, and a volume of 2.2×10^5 m^3. The mean depth of the lake is 4.4 m and volume development is 1.32. A single outflow stream leaves the lake, flowing over a steep bank into nearby lake 305. There are no inlet streams, and most inflow occurs during snowmelt, or during occasional heavy rainstorms.

The lake drains a watershed of 49 hectares covered primarily by black spruce (*Picea mariana*) and jackpine (*Pinus banksiana*) forest in weakly developed brunisols overlying Precambrian granite. Details on morphometry, vegetation, and geography of the area are given by Brunskill and Schindler (1971). Other data have been published on chemical and physical properties (Armstrong and Schindler 1971; Schindler 1971); periphyton (Stockner and Armstrong 1971); zooplankton (Patalas 1971); zoobenthos (Hamilton 1971); diatom stratigraphy in sediments (Stockner 1971); and sediment chemistry (Brunskill et al. 1971). The lake is very low in electrolytes (surface water was 21 μmhos/cm at 25 C before fertilization experiments were begun) and slightly colored (20–25 Pt units).

Vernal mixing has not been complete in the 4 years we have observed the lake, due to the protected nature of the small basin. For example, when ice left the lake on April 23, 1969, a hot still day, thermal stratification was pronounced by midafternoon, even though oxygen was only 4.47 ppm in surface water and completely absent below 9 m (Fig. 1). The lower part of the hypolimnion appears to be aerated once each year, during fall overturn (Fig. 2). The thermocline of the lake is usually shallow, 3–4 m in depth, during the summer (Schindler 1971a fig. 6*f*). Samples from the anaerobic hypolimnion smelled of H_2S.

Materials and Methods

Sample Collection

Samples in 1969 were taken with a van Dorn bottle (usually at depths of 1, 3, 5, 7, and 9 m). Frequency of sampling was every 2nd week in the ice-free season, and monthly under ice. All samples were taken just before the weekly fertilization was performed (see below).

Analyses

Oxygen was determined by the azide modification of the Winkler method in Standard Methods (American Public Health Association 1965). Total carbon dioxide was measured by gas chromatography (Schindler and Holmgren 1971). Unfiltered samples were acidified by addition of 1 cc conc. HCl per 100-cc sample for later determination of calcium, magnesium, sodium, and potassium by atomic absorption spectrophotometry.

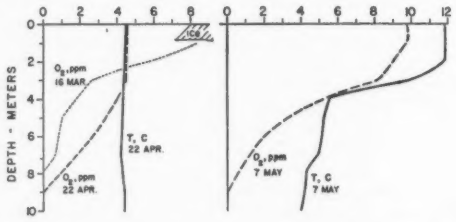

FIG. 1. Oxygen and temperature profiles the day after ice-out, April 22, 1969, and 2 weeks later.

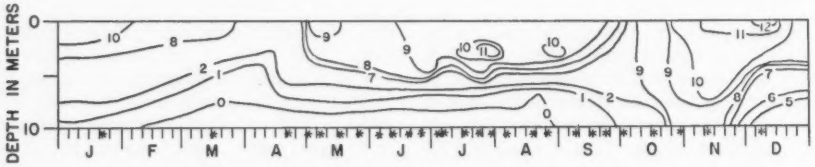

FIG. 2. Dissolved oxygen, ppm, 1969. * indicates dates of sampling.

A 500-cc sample was filtered on a previously ignited Whatman GF/C filter. The filter was then dried in a desiccator over silica gel, and stored at –10 to –20 C until analysis of suspended particulate matter. The filter was cut in half to give equal portions of the collected suspended matter. Carbon and nitrogen in one-half were determined with a Perkin-Elmer Model 240 Elemental Analyzer, and phosphorus in the other by a spectrophotometric molybdenum blue method after ignition at low temperature and acid extraction.

The filtrate was kept for nutrient analyses. Ammonium nitrogen was determined by the phenol-hypochlorite method of Solorzano (1969), nitrite by the method of Bendschneider and Robinson (1952), nitrate by cadmium/copper reduction to nitrite (Wood et al. 1967), phosphate by the molybdate/antimony/ascorbic acid method of Murphy and Riley (1962), and total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) after ultraviolet photochemical combustion (Armstrong and Tibbitts 1968). The photochemical combustion method gave erroneously low values for total dissolved nitrogen in hypolimnetic waters during summer stagnation due to incomplete oxidation of the high concentrations of ammonia present.

A second 500-cc sample was filtered for plant-pigment determination, using a 4.25-cm diam Whatman GF/C filter and $MgCO_3$. Pigments were extracted by 90% acetone with the aid of a Ten Meulen tissue grinder and determined fluorometrically (Yentsch and Menzel 1963). The fluorometer was standardized with acetone extracts of phytoplankton in which plant pigments had been determined spectrophotometrically. The results are expressed as chlorophyll *a*, although we are aware that substantial proportions of phaeophytins or phaeophorbides were sometimes present.

Color of filtered water samples was measured in Hazen units, using a visual comparator (Tintometer Company), with glass disc standards.

Plankton

Phytoplankton production measurements were begun in May 1969. Production was measured by the ^{14}C method (Schindler and Holmgren 1971). Biweekly incubations were made during the ice-free season, with two light bottles and one dark bottle at each of seven depths (0, 0.5, 1, 2, 3, 5, and 7 m). Exposure was for 4 hr at midday. Results were corrected for filtration error (Arthur and Rigler 1967; Schindler and Holmgren 1971).

Phytoplankton standing crop was sampled at the same depths as production measurements. Samples were preserved in Lugol's solution and an inverted microscope was used for counting (Utermöhl 1958).

Chemical Additions

Additions were made weekly for 17 weeks, starting on June 26 and ending on October 16. Each addition was made at 11:00 AM, just after routine chemical sampling was completed and primary production incubations begun. 90.7 kg (200 lb) of sodium nitrate, and 4.54 kg (10 lb) of disodium hydrogen phosphate, both of "Food Grade" quality, were used. Each addition corresponded to an increase of 68 μg N/liter, 4.5 μg P/liter, and 0.12 mg Na/liter for the lake as a whole. The total addition in the 17 weeks was 5.04 g N/m², 0.34 g P/m², and 8.9 g Na/m². These rates of nutrient addition are roughly similar to those reaching the lower St. Lawrence Great Lakes (see Discussion).

We consider the usual method of adding fertilizer to lakes, by throwing in the granular material, to be unsatisfactory, since undissolved fertilizer has sometimes been observed on the bottom (Brook and Holden 1957; Smith 1969; Weatherly and Nichols 1955). The following procedure was therefore used. The chemicals were placed directly into a 12-ft fiberglass boat, which had a 9.5 hp motor. The drainplug in the transom was then removed, the boat was allowed to become one-third filled with water, and the contents were stirred with a paddle to dissolve as much material as possible. The boat was then driven around the lake with the plug removed, fast enough to empty the contents into the wake by Venturi effect. A piece of wire screening at the back of the boat was used to prevent large undissolved particles from being washed out. The process was repeated from three to six times, taking 30–90 min altogether, depending on the water temperature.

Samples were taken from a number of stations within an hour of fertilization on three occasions, and nitrate determined to see how uniform the distribution was. The coefficients of variation for 10 samples were ±17, 26, and 33%. On October 16 the water temperature was too low to dissolve the disodium hydrogen phosphate and an equivalent quantity of phosphoric acid was substituted.

Tube Experiments

The response of phytoplankton standing crop to various nutrient regimes was tested in tubes made of polyethylene "layflat" tubing of doubled width 1.65 m (5 ft) and thickness 0.10 mm (0.004 inches). The upper end of each tube was held open by a hoop 1.0 m in diameter of polyvinyl water pipe, which was supported on a wooden frame with floats (Fig. 3). The lower end was weighted with a hoop of iron bar sealed into the hem and allowed to sink into the sediments.

Set 1 — enrichment with carbon

When total CO_2 concentrations fell to almost undetectable levels in July and August of 1969 (see results), this set of four tubes was set up first to see whether shortage of carbon was affecting phytoplankton growth. A single large dose of carbon was added to each tube in the absence of nitrogen and phosphorus, using organic, inorganic, and gaseous carbon sources. Compounds added and quantities are given in Table 1. The experiment was begun on August 4.

Set 2 — enrichment with phosphorus, nitrogen, and carbon in combination

This set of eight tubes was begun on August 22. Carbon, nitrogen, and phosphorus were added in various combinations as single large doses. The additions to these tubes are also shown in Table 1.

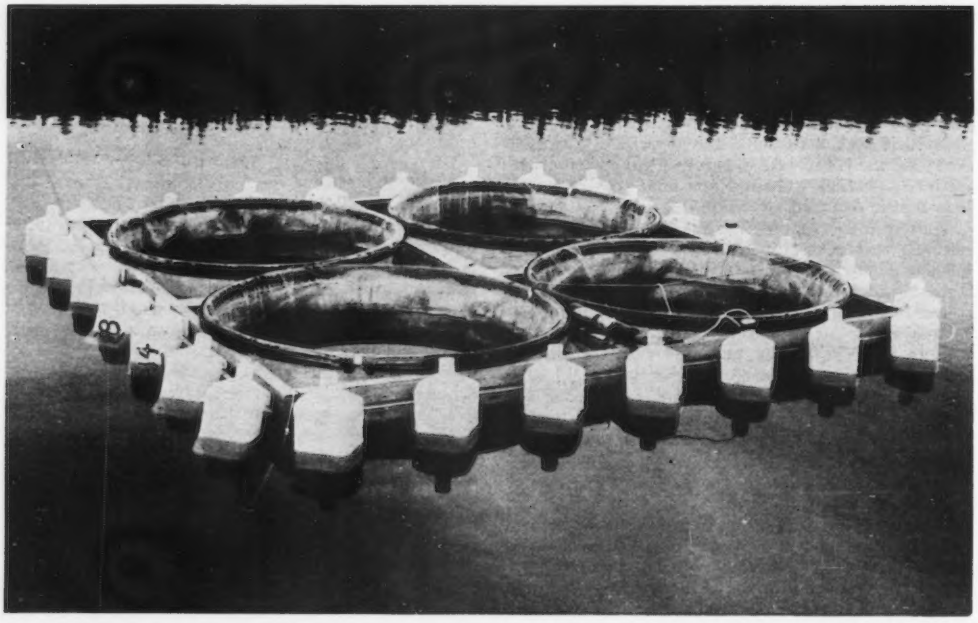

FIG. 3. Polyethylene tubes used for enrichment experiments. Tube diameter is 1.0 m and length is 10 m. The ring at the top is of polyvinyl water pipe. The lower end of the tube is held in the mud by a ring of ½-inch iron bar sealed in the hem of the tube.

TABLE 1. Chemical additions in tube experiments.

Set no.	Date (*1969*)	Tube	Additions
1	Aug. 4	1	Control (no additions)
		2	CO_2 (bubbled in slowly with three aquarium stones at 9 m for several days)
		3	Sodium acetate (≡ 1500 μmole C/liter)
		4	Sodium bicarbonate (≡ 1500 μmole C/liter)
2	Aug. 22	5	Control (no additions)
		6	0.714 g urea [a] +
		7	0.072 g $(NH_4)_2HPO_4$ + 0.705 g urea
		8	0.072 g $(NH_4)_2HPO_4$ + 0.705 g urea + 84 g $NaHCO_3$
		9	0.031 g H_3PO_4 + 84 g $NaHCO_3$
		10	0.031 g H_3PO_4
		11	2 liters urine[b]
		12	2 liters urine + 84 g $NaHCO_3$

[a] $CO(NH_2)_2$.

[b] Urine (from residents of the ELA camp) contained approximately 1.5 g/liter of total dissolved phosphorus and 3.6 g/liter of total dissolved nitrogen.

All chemicals were added by dissolving them and stirring them into the epilimnion of each tube (a depth of 2–3 m) with a paddle. Phytoplankton and water chemistry were followed for several weeks, using samples taken with a 3-m length of 1-inch diam tubing lowered into the epilimnion of the tube (Lund and Talling 1957).

Results

PHYTOPLANKTON SPECIES AND STANDING CROP

Phytoplankton standing crop increased by several times after fertilization, as shown by chlorophyll *a* isopleths (Fig. 4) and phytoplankton cell volume (Fig. 5 and 6). Midsummer chlorophyll *a* concentrations in 1968, the year before fertilization, did not exceed 3 μg/liter. In September 1969, after 12–14 weeks of fertilization, a maximum of 51.8 μg/liter chlorophyll *a* was reached. Summer standing crops for five nearby unfertilized lakes (no. 239, 240, 303, 304, and 305) in 1969 ranged from 0.6 to 20.3 μg/liter chlorophyll *a*. This range includes one abnormally high value for lake 303. If this sample is excluded, the range was 0.6–8.6 μg/liter.

The phytoplankton species typical for small unfertilized lakes in the area were present in lake

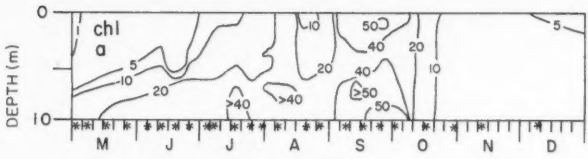

FIG. 4. Chlorophyll *a* (uncorrected for phaeophyton), μg/liter, 1969.

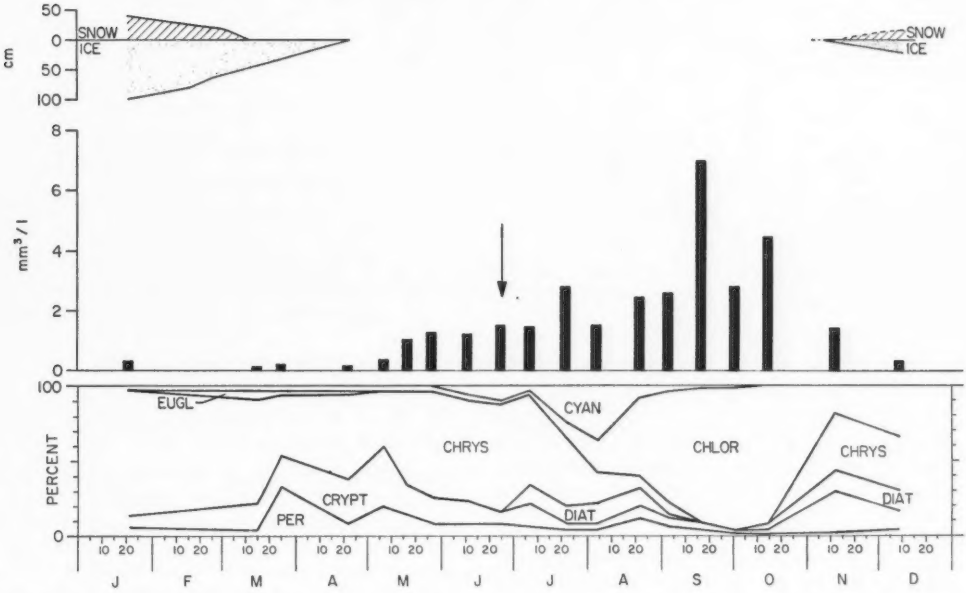

FIG. 5. Average phytoplankton volume in the euphotic zone, lake 227, 1969. The arrow indicates the date of the first fertilization.

227 before fertilization. In 1968, prior to fertilization, *Cryptophyceae* (chiefly *Cryptomonas* spp.) were dominant throughout the summer, whereas the diatoms *Cyclotella* and *Tabellaria* and chrysophyceans *Chromulina* sp. and *Kephyriopsis* spp. were important in June. Chlorophytes and cyanophytes were never abundant, although several species were present in small numbers.

Species present in the winter before fertilization were also typical for unfertilized ELA lakes, although standing crops were slightly higher than average (S. K. Holmgren and H. J. Kling unpublished data). *Chrysochromulina parva* Lackey v. and *Botryococcus braunii* Kg. were dominant species.

After ice left the lake in May, 1969, chrysophyceans and cryptomonads increased rapidly. Small forms of the genera *Ochromonas*, *Chromulina*, and *Pseudokephyrion* were dominant. By late May, when nutrients in the epilimnion were depleted, a metalimnetic maximum had begun to form. A small *Ochromonas* sp. bloomed at 5 m, whereas *Cryptomonas* spp. were numerous at 7 m. These forms appeared to be adapted to low light. In June, a third population, filamentous blue-greens (*Oscillatoria*, *Spirulina*), appeared at 7 and 9 m. By late June, just before fertilization, *Ochromonas* sp. mentioned above was partly replaced by *Dinobryon sertularia* v. *protuberans* (Lemm.) Kg.

Phytoplankton species changed drastically after fertilization. An increase in diatoms (*Tabellaria*, *Rhizosolenia*, *Synedra*, and *Cyclotella*) was the first change to follow (July 7 samples). *Mallomonas pumilio* Harris and Bradley v. and other small chrysophyceans had increased at 1 m, whereas *Dinobryon* dominated at 5 m. The vertical distribution of chrysophyceans was bimodal (Fig. 6).

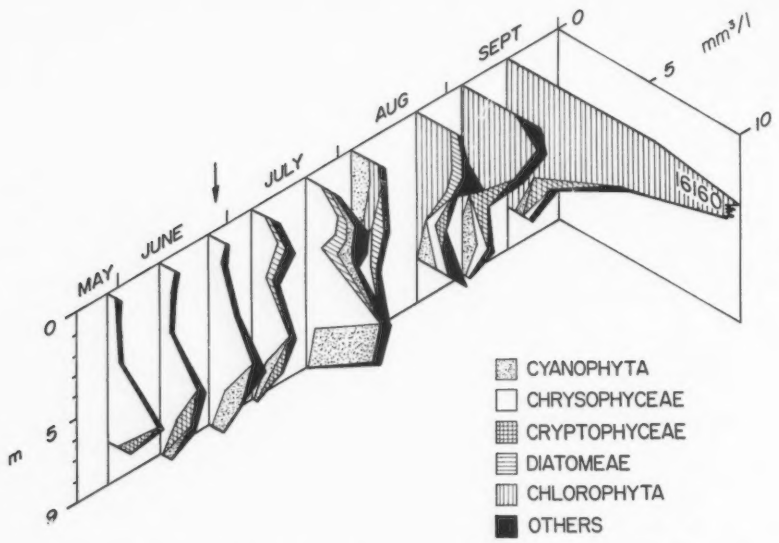

FIG. 6. Seasonal changes in the vertical distribution of phytoplankton, lake 227, 1969.

No floating water blooms were observed at any season in 1969. In late July, a small blue-green alga, *Pelogloea bacillifera* Ltb. increased in abundance in the epilimnion, and *Oscillatoria* and *Spirulina* increased rapidly in the metalimnion. By early August, chrysophyceans had almost entirely disappeared, leaving the chlorophyte genera *Phacomyxa*, *Spondylosium*, and *Staurastrum* as dominants. None had been important in 1968 samples.

By late August and September, Cyanophyta and diatoms also disappeared from the epilimnion, leaving only green algae as dominants. In the metalimnion, *Cryptomonas*, *Dinobryon*, *Oscillatoria*, and *Spirulina* still persisted. On September 17, the largest average biomass of the year was recorded, almost 7000 mg/m^3, over 95% green algae of the above-mentioned genera.

In November, 3 weeks after fertilization had ceased, chrysophyceans and cryptomonads again surpassed Chlorophyta in biomass. Small *Chromulina* and *Ochromonas* again dominated, but *Mallomonas pumilio* v., *Rhodomonas minuta* Skuja, *Cryptomonas obovata* Skuja, and *C. pusilla* Bachm. were also common.

After freeze-up, chrysophyceans became the largest group, and green algae decreased even further.

A complete list of phytoplankton identified from the lake is on file at the Freshwater Institute.

PHYTOPLANKTON PRODUCTION

Contrary to expectations, an increase in primary production did not accompany the postfertilization increase in phytoplankton standing crop. Highest production was recorded in the early spring, just after ice left the lake and before fertilization was begun (Fig. 7). Average daily values during the ice-free season were similar to those obtained in unfertilized lakes of similar morphometry. Schindler (1972 table 3) gives comparative data. The fact that routine production measurements were always made just before weekly nutrient additions, when minimum ^{14}C uptake would be expected, may be partly responsible. Additional tests were performed in 1970 (D. W. Schindler et al. unpublished data).

WATER CHEMISTRY

Even though enough phosphate was added to increase concentrations by 10 µg P/liter per week, molybdate-reactive phosphorus was undetectable on almost all occasions (Fig. 8), as it had been throughout 1968. Most of the added phosphate was assimilated by particulate matter, presumably by phytoplankton, although slight possible increases in total dissolved phosphorus (TDP) were also detected (Fig. 9 and 10). Prefertilization levels of

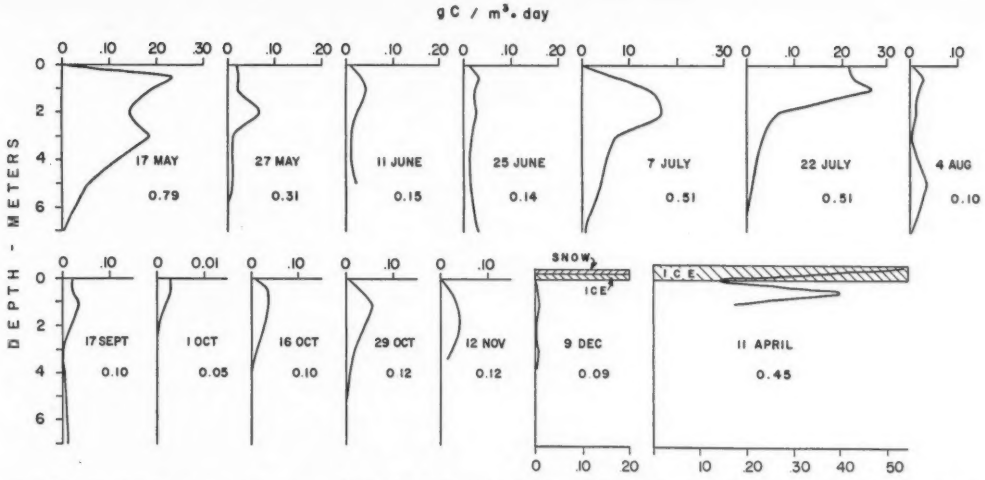

FIG. 7. Production measurements made by the ^{14}C method, May 1969–April 1970. The point shown in the ice on April 11, 1970, represents a bloom of *Dinobryon* that occurred in water between two layers of ice. Numbers below panels represent total production, mg C/m² per day.

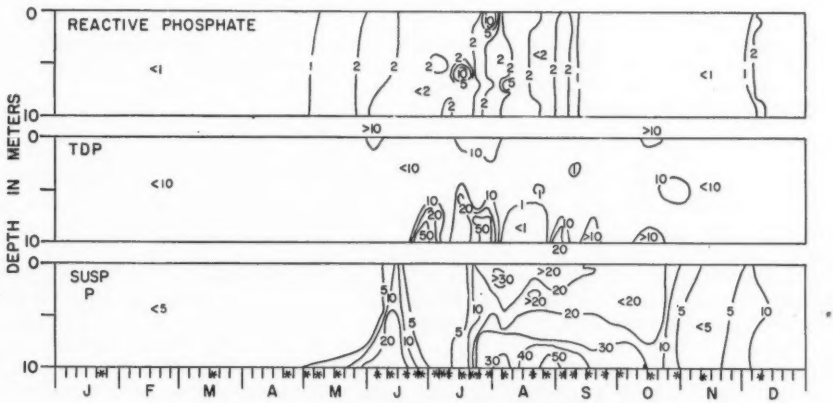

FIG. 8. (*top*) Reactive phosphate, μg/liter, 1969.
FIG. 9. (*middle*) Total dissolved phosphorus (TDP), μg/liter, 1969.
FIG. 10. (*bottom*) Suspended particulate phosphorus, μg/liter, 1969.

TDP and particulate P were 3–5 and 1–5 μg/liter, whereas maximum levels during fertilization in 1969 were 2–8 and 15–29 μg/liter, respectively.

The added nitrate was also quickly assimilated by particulate matter (Fig. 11). Particulate nitrogen peaked at 320–600 μg/liter in late summer, compared to prefertilization concentrations of 50–100 μg/liter. Contrary to findings for phosphorus, however, nitrate concentrations increased somewhat after fertilization, often exceeding 100 μg N/liter in late summer (Fig. 12), whereas 1968 nitrate concentrations were only 0–10 μg/liter. Since 150 μg/liter of NO_3-N were added weekly, only a very small percentage remained. Total dissolved nitrogen (TDN) in the epilimnion also increased during fertilization, ranging from 200 to 300 μg/liter in the summer of 1969 (Fig. 13) compared to 130–160 μg/liter before fertilization. Ammonia was scarce

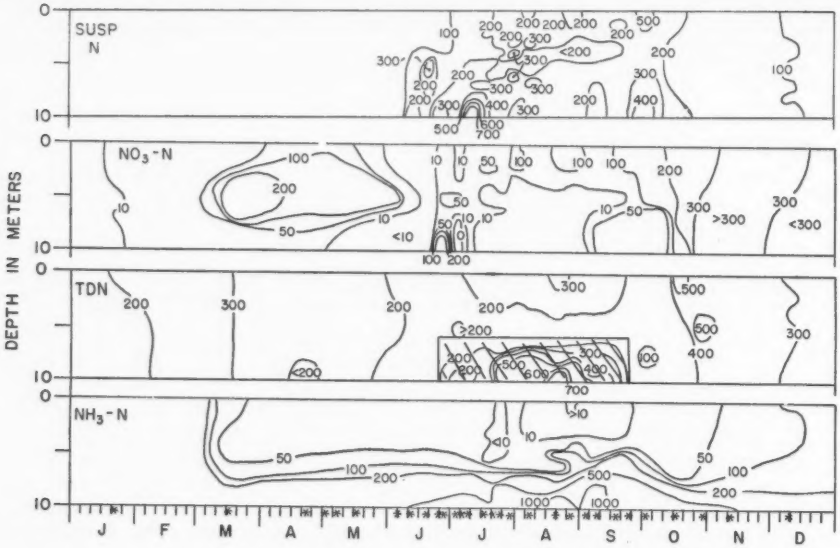

FIG. 11. (*top*) Suspended particulate nitrogen, μg/liter, 1969.

FIG. 12. (*second*) NO_3:-N, μg/liter, 1969.

FIG. 13. (*third*) Total dissolved nitrogen (TDN), μg/liter, 1969. The hatched area indicates that data were unreliable due to incomplete photooxidation of the high levels of ammonia present. Later (1970) data indicate that the total dissolved nitrogen in the hypolimnion in midsummer is almost entirely NH_3; therefore, the hypolimnetic concentrations of NH_3 shown in Fig. 14 are very close to those for total dissolved nitrogen.

FIG. 14. (*bottom*) NH_3-N, μg/liter, 1969.

in the epilimnion during the summer both before and after fertilization, although large amounts accumulated in the hypolimnion during summer stratification and were brought into circulation during fall overturn (Fig. 14). This is common in shallow (maximum depth <12 m) lakes of the area. Nitrite concentrations did not exceed 2 μg N/liter during the year.

Total CO_2, which is normally very low in the epilimnions of small ELA lakes in midsummer due to phytoplankton production (D. W. Schindler unpublished data) was even more severely depleted in lake 227. The lowest values recorded in 1969 (<20 μmoles/liter) occurred August 3. Total CO_2 in the hypolimnion increased throughout the summer (Fig. 15). Suspended carbon increased after enrichment, paralleling increases in suspended phosphorus and nitrogen (Fig. 16). The autumnal decrease in suspended carbon also corresponded to similar changes in suspended nitrogen and phosphorus.

The *p*H of the epilimnion increased sharply within a few weeks after fertilization was begun (Fig. 17). Experiments conducted in 1970, including direct measurement of P_{CO_2}, proved that CO_2 depletion by algae alone was responsible (D. W. Schindler et al. unpublished data). Values returned to normal (*p*H 6–7) after fall turnover.

Sodium concentrations in the epilimnion were about three times higher at the end of summer stagnation in 1969 than at any time before fertilization, as one might expect, since sodium salts of both nitrogen and phosphorus were used. There was no detectable movement of sodium into the hypolimnion until fall turnover (Fig. 18).

No striking changes were noted in other major cations after fertilization. Calcium (Fig. 19) was depleted in the epilimnion and enriched about three times in the hypolimnion during summer stagnation. Slight increases in magnesium and potassium in the hypolimnion were observed during the same period (Fig. 20 and 21). Such patterns are common in ELA lakes (F. A. J. Armstrong and D. W. Schindler unpublished data).

Silicate concentrations remained high and relatively constant throughout the year (Fig. 22), which is not surprising since diatoms were not abundant in the plankton.

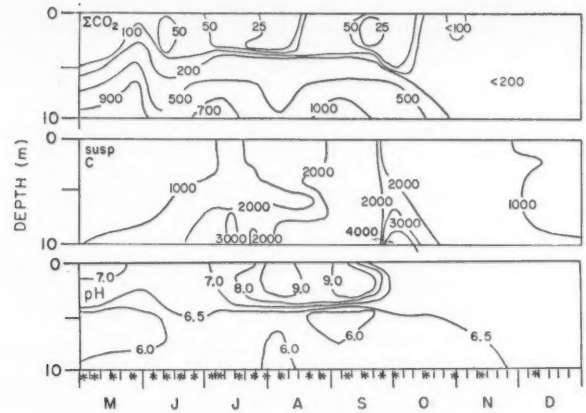

FIG. 15. (*top*) Total CO_2, μmoles/liter, 1969.
FIG. 16. (*middle*) Suspended particulate carbon, μg/liter, 1969.
FIG. 17. (*bottom*) Seasonal changes in *p*H, 1969.

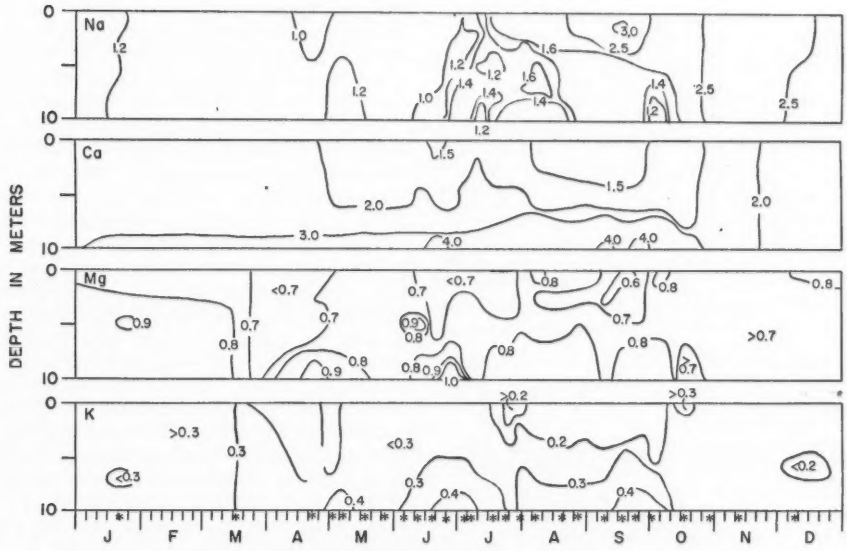

FIG. 18. (*top*) Na^+, mg/liter, 1969.
FIG. 19. (*second*) Ca^{++}, mg/liter, 1969.
FIG. 20. (*third*) Mg^{++}, mg/liter, 1969.
FIG. 21. (*bottom*) K^+, mg/liter, 1969.

DESTINATIONS OF THE NUTRIENT ADDITIONS

In the absence of complete hydrological data for the year, only gross results of enrichment will be given here. A week-by-week analysis of the destination of added P and N will be reserved for a later paper.

A natural nutrient budget was estimated, using meteorological data from the area and hydrological

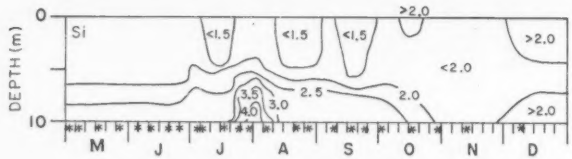

FIG. 22. Dissolved silicate, μg Si/liter, 1969.

and chemical data from nearby lakes 239, 240, 303, and 304.

During the period June 26 to October 16, when weekly enrichments were made, 415 mm of precipitation fell in the area (data from Teggau Lake station, 4 miles to the southwest). Discharge from the drainage system, which includes lakes 239, 240, 303, and 304, was equal to 68% of precipitation during the period, so that evaporation and evapotranspiration accounted for 32% of the water losses from the drainage (Newbury and Cherry 1970). By assuming that evaporation and evapotranspiration were equal, a rough estimate of natural nutrient supply was obtained, using the formula:

$$S = (p)\,(A_o)\,(C_1) + (p\text{–}E)\,(A_d)\,(C_2), \qquad (1)$$

where S is the natural supply (g/m² per year) of each nutrient, A_o is the surface area of the lake in m², C_1 is nutrient (N or P) concentration in rainfall in g/m³, A_d is the area of terrestrial drainage in m², p is the precipitation, E is the evapotranspiration from the basin, both in meters, and C_2 is the nutrient concentration in runoff. C_1 and C_2 are assumed to be equal to values found for rainfall and runoff on lake 239 during the period (Armstrong and Schindler 1971), and A_o and A_d are taken from Brunskill and Schindler (1971). Solving the equation using C_1 and C_2 values for N and P:

$$S_P = (0.615)(5 \times 10^4)(0.018) + (0.153)(49 \times 10^4)(0.0064)$$

or 1033 g of P; 553 as precipitation and 480 as runoff.

$$S_N = (0.615)(5 \times 10^4)(0.378) + (0.153)(49 \times 10^4)(0.198)$$

or 26,423 g of N; 11,623 as precipitation and 14,800 as runoff.

The C_1 and C_2 given above are for TDN and TDP. These must be regarded as minimum estimates, since nitrogen and phosphorus also enter the lake with particulate material. Assuming that the original source of N and P to both terrestrial drainage and lake surface is the atmosphere, and that input equals output for the terrestrial basin (i.e., that it is in equilibrium), the total supply to the lake (including particulate matter) should equal:

$$S = (p)(C_1)(A_d + A_o)$$

$$\text{or } S_P = (0.615)(0.018)(54 \times 10^4) \text{ or } 5940 \text{ g P} \qquad (2)$$

and

$$S_N = (0.615)(0.378)(54 \times 10^4) \text{ or } 125{,}280 \text{ g N.}$$

Although equation 2 may give estimates closer to total nutrient supplied from the watershed, it is undoubtedly an overestimate of nutrient useful to phytoplankton, since much of the allochthonous particulate matter must sediment without permanently entering biological cycles in the water column of the lake at all. We shall therefore base all further discussion on solutions to equation 1.

Natural nutrient input was insignificant when compared to the artificial supply to the lake; 6% of the total input for phosphorus and 10% for nitrogen (Fig. 23).

The fate of all nitrogen and phosphorus entering the lake during the period of fertilization was also calculated. On October 16, the last date the lake was enriched, only 27% of the total seasonal phosphorus income remained in the water column. Judging by outflow volumes and concentrations of total dissolved and suspended phosphorus in epilimnetic waters, 11% was lost via the outlet of the lake, leaving 62% unaccounted for. This quantity was presumably lost to the sediments. The corresponding figures for nitrogen were: water column, 42%; outflow, 14%; presumed lost to sediment, 44%.

It is of interest to compare the fate of nitrogen and phosphorus in the lake with that of the sodium added with the fertilizer. Sodium was added far in excess of biological requirements, and since it is highly soluble, little or no loss to sediments is likely. During the period from June 26 to October 16, 443 kg of Na were added to the lake. An additional 81 kg were added by rainfall and runoff, again based upon inference from measurements made in the basin of lake 239. The mass present in lake 227 on June 25 was 232 kg, and that on October 16, 566 kg, leaving 190 kg of Na (= 36%), which was presum-

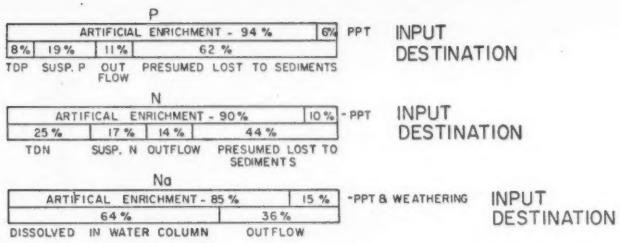

FIG. 23. Sources of input and destinations of phosphorus, nitrogen, and sodium to lake 227 during the period of fertilization, June 26 to October 16, 1969. Total inputs were: P, 18 kg; N, 262 kg; Na, 520 kg.

ably lost via the outflow (Fig. 23). The quantity of sodium lost, calculated from sodium concentrations and outflow volume per square meter of drainage during the period of fertilization (hydrological data for nearby drainages of lakes 239, 240, 303, and 304; see Newbury and Cherry 1970), would be 43%, which agrees well enough for present purposes.

TUBE EXPERIMENTS

Set 1 — enrichment with carbon

Phytoplankton standing crop, as measured by chlorophyll *a*, decreased in all tubes, including the unfertilized control, throughout the experiment. Chlorophyll in the lake, where weekly phosphorus and nitrogen additions were continued, increased throughout the same period (Fig. 24). The dominant phytoplankton species were similar to those in the lake, chiefly *Staurastrum* and *Spondylosium* spp. (Fig. 24, Table 2). Periphyton on the walls of the tubes did not become abundant until near the end of the experiment.

Total CO_2 concentrations in the tube bubbled with gaseous CO_2 remained slightly higher than the control (Fig. 24). The experiment in this tube was terminated accidently after 32 days when the bottom of the tube broke loose from the sediments. In the acetate-enriched tube, CO_2 concentrations increased considerably between 25 and 37 days after enrichment, possibly due to microbial breakdown of the acetate. In the bicarbonate-enriched tube, total CO_2 decreased throughout the experiment.

Set 2 — enrichment with carbon, nitrogen, and phosphorus

Only tube 6, which received no phosphorus, did not develop larger phytoplankton standing crop than the control tube (Fig. 25). Highest phytoplankton standing crops were found in tubes receiving phosphorus plus nitrogen or carbon, or both. The added phosphorus and nitrogen disappeared quickly, reaching undetectable levels by the first sampling date, 1 week after addition. On the contrary, CO_2 added as bicarbonate did not return to prefertilization levels for about 40 days (Fig. 25).

The final two tubes of set 2, enriched with urine, showed convincingly that high doses of nutrients can quickly produce enormous standing crops of phytoplankton, even in naturally unproductive lakes (Fig. 26). Carbon, nitrogen, and phosphorus supplied to the tubes were depleted rapidly, reaching preenrichment concentrations by the end of the experiment. The phytoplankton of both tubes was dominated by Chlorophyta, primarily a large *Chlamydomonas* species not found in the lake or in other tubes (Table 2).

Apparently, from the above two sets of experiments, phosphorus was required as a primary stimulus for phytoplankton increase in the tubes. Nitrogen or carbon may have caused additional increases in phytoplankton once the phosphorus supply was adequate.

Discussion

SIGNIFICANCE OF CHANGES IN CHEMISTRY AND PHYTOPLANKTON

The concentration of inorganic phosphorus in lake water has often been used as a basis for arguments about the role of phosphorus in eutrophication, both by proponents and opponents of the theory that algal growth may be controlled by the input of phosphorus to lakes.

Sawyer (1947), on the basis of chemical analyses of Wisconsin lake waters, including several lakes where blooms were present, predicted that algal blooms would appear whenever late winter concentrations of molybdate reactive phosphorus exceeded 10 μg/liter or dissolved inorganic nitrogen exceeded 300 μg/liter.

Lange (1967), Kuentzel (1969), and Norman (1970) argued that since minute concentrations of phosphate often occur during algal blooms, the requirements for this ion by algal water blooms

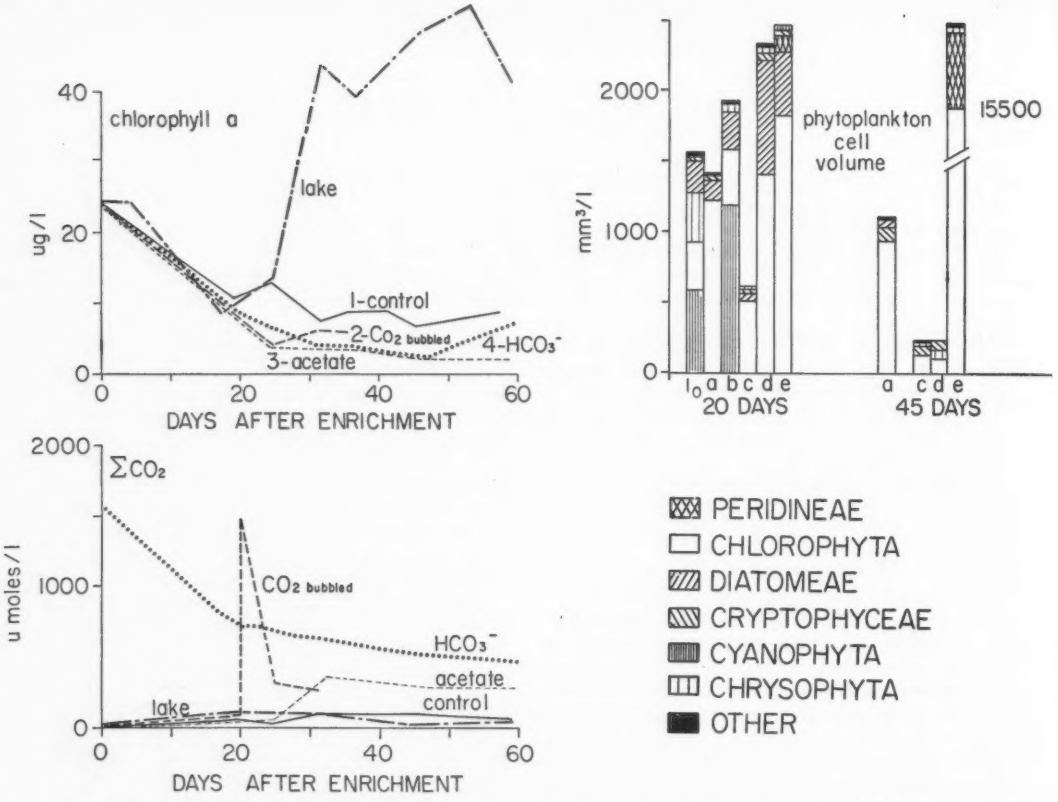

FIG. 24. Results of tube enrichment set 1 (enrichment with carbon). Letters on phytoplankton cell volume panel are keyed as follows: l_o, phytoplankton in the lake and tubes on August 4, when the experiment was begun; *a–e*, phytoplankton after 20 and 45 days. *a*, Tube 1 — control (no additions); *b*, tube 2 — CO_2 bubbled; *c*, tube 3 — enriched with sodium acetate (= 1500 μmoles C/liter); *d*, tube 4 — enriched with sodium bicarbonate (= 1500 μmoles C/liter); *e*, phytoplankton in the lake, which was enriched weekly with P and N at rates described in the text. Tubes were enriched only once, at the start of the experiment. Amounts of nutrients added are given in Table 1.

are either too low to be controllable or are not limiting to the plankton.

Our results demonstrate that the amount of phosphorus *supplied*, rather than the amount in solution at a given time must be known to predict algal growth. Although the amount of phosphorus added to lake 227 each week was sufficient to increase the concentration of phosphorus in the epilimnion of the lake by approximately 10 μg/liter, molybdate reactive phosphate was undetectable (<1 μg/liter) within an hour after fertilization. The concentration of dissolved phosphate is therefore an unsatisfactory index for the role of this element in eutrophication.

Einsele (1941; see also Hutchinson 1957 p. 745) conducted a phosphate fertilization experiment that gave results similar to ours. He added 14 kg of superphosphate–phosphorus to Schleinsee, a small eutrophic lake that contained detectable molybdate reactive phosphate only at overturn. The added phosphorus was rapidly taken up and sedimented as sestonic phosphorus. Nearly half of the added phosphorus disappeared from the epilimnion in 2 days. In the following year (1938), two additions, each of 47 kg superphosphate–phosphorus, were made to the same lake, yielding similar results. At no time in either experiment did any great amount of the added phosphorus appear in the hypolimnion. By

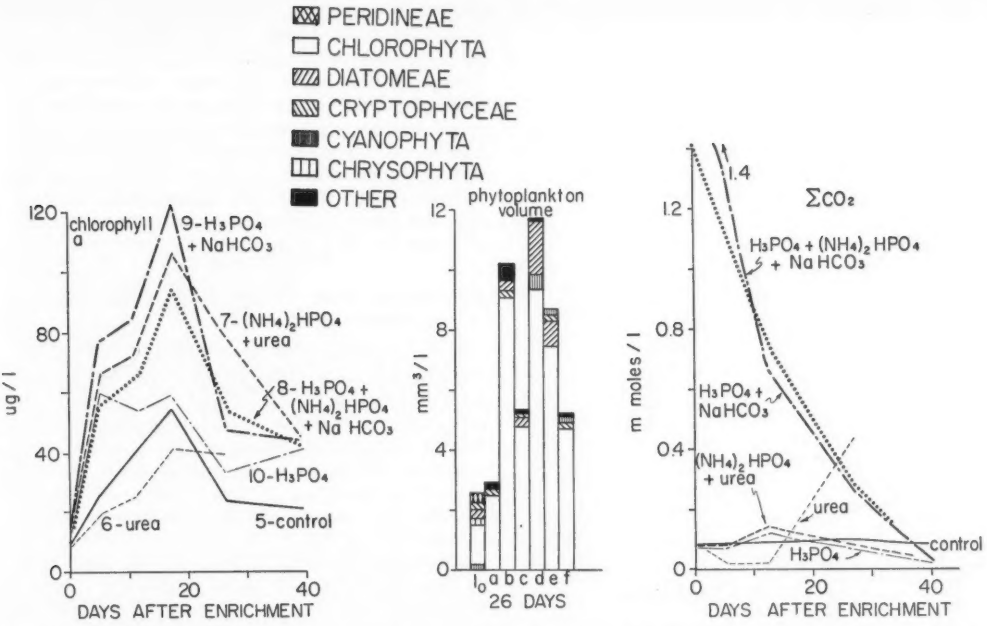

FIG. 25. Results of tube enrichment set 2 (enrichment with P, N, and C in various combinations). Amounts of chemicals added are given in Table 1. Letters on the phytoplankton volume panel are keyed as follows: l_o, phytoplankton in the lake on August 22, the day the experiment was begun; *a*, tube 5 — control (no additions); *b*, tube 6 — ammonium phosphate + urea; *c*, tube 7 — urea; *d*, tube 8 — ammonium phosphate, urea, and sodium bicarbonate; *e*, tube 9 — phosphoric acid and sodium bicarbonate; *f*, tube 10 — phosphoric acid. Tubes were enriched only once, at the start of the experiment. Amounts of nutrients added are given in Table 1.

late 1939, the phosphorus content of the lake had returned to normal values, and no permanent effect on the biochemistry of the lake was noticeable, a fact that Hutchinson (1957 p. 746) calls one of the most important discoveries ever made in limnology. Since the fate of added phosphorus is similar both in an originally unproductive lake (lake 227 was oligo- to meso-trophic, based on the phytoplankton standing crop criteria of Winberg (1963) and Sakamoto (1966)) and a eutrophic one (Einsele 1941), complete removal of external sources of phosphorus to a lake would probably allow it to return rapidly to its natural steady-state condition.

Hutchinson (1957 p. 735) remarked that lakes with clinograde oxygen curves tended to show a considerable increase in phosphorus concentrations of the lower hypolimnion. Although this hypolimnetic increase appeared in lake 227, only TDP and particulate P were involved, as there was no corresponding increase in hypolimnetic reactive phosphate (Fig. 8–10). The increase can be entirely accounted for by sedimenting seston.

Mortimer (1941, 1942), working in Esthwaite Water and in anaerobic tanks, found that ferrous iron appeared in the lake water when the redox potential dropped below 0.2 v. Molybdate reactive phosphate was released from the sediments during this period. Since in situ redox potential measured at the mud–water interface in the center of lake 227 in August was less than –0.2 v (measured with a Radiometer 4 *p*H meter equipped with a platinum-calomel electrode pair and long shielded leads), and since the phosphorus concentration of sediments in lake 227 was higher than either Esthwaite Water or Linsley Pond (Brunskill et al. 1971 tables 4, 7, and 12) both of which are reported to have high rates of phosphate release from sediments in late summer (Hutchinson 1957 p. 736), it is impossible to explain why no phosphate is released from sediments in lake 227 by the widely believed theories of Hutchinson (1957), Mortimer (1941, 1942), and Einsele (1938). Hayes (1964) expressed doubts about the generality of the redox potential theory, and pointed out that chemical reactions in sediments may be

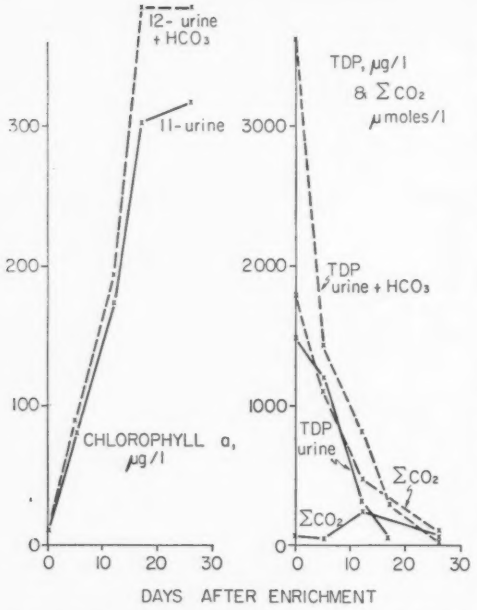

FIG. 26. Results of enrichment with urine (tube 11), and urine + bicarbonate (tube 12). Enrichment procedure is described in the text; solid line, urine; broken line, urine + bicarbonate.

the result of microbial processes as well as geochemical ones.

Over 80% of total iron in sediments of lake 227 can be extracted with dilute acid, yet less than 15% of total phosphorus appears in this extract (Brunskill et al. 1971). Possibly sediment P is associated with a precipitate that is not affected by redox potential, i.e., organic mater or, $AlPO_4 \cdot nH_2O$. In the monimolimnions of meromictic lakes of ELA, where anaerobic conditions are present throughout the year with Eh < -200 mv, little or no molybdate reactive phosphate is detected (D. W. Schindler and P. Campbell unpublished data).

Some authors (Kratz and Myers 1955; Provasoli 1958; Wetzel 1966; Benoit 1969) have predicted that major ions will limit algal production, particularly in uncontaminated lakes. If this prediction were true, one would expect the small concentrations of these ions in lake 227 to be severely depleted in the euphotic zone, particularly after phytoplankton had already been stimulated by addition of nitrogen and phosphorus. As Fig. 18–21 demonstrate, no major cation was depleted by more than 33% in the euphotic zone of lake 227 during summer stagnation. Similar results were found in unfertilized lakes 239, 240, 304, and 305. The large amounts of sodium added to lake 227 did not cause an increase in hypolimnetic concentrations of this element during summer stagnation, as one would expect to result from sedimentation and decomposition of plankton if the element was required in large quantities by phytoplankton. Barrett (1957) found no evidence that potassium added to several small Michigan lakes stimulated phytoplankton production. The lakes contained low initial concentrations of potassium (0.2–2.0 ppm).

Comparison with Other Enrichment Studies in Shield Waters

Both Langford (1950) and Smith (1969) concluded that phosphorus was the primary limiting nutrient in their studies. Since ELA, Algonquin Park, Ont., and Crecy Lake, N.B., represent western, central, and eastern regions of the Canadian Precambrian Shield, it seems safe to conclude that phosphorus limitation of algal growth is widespread in culturally undisturbed areas of this geological formation.

Although total CO_2 in lake 227 in summer is among the lowest recorded for lakes anywhere in the world, no indication of carbon limitation, at least in the 1st year of fertilization, has been observed. Smith (1969) recorded a phytoplankton maximum within 1 week after fertilization with salts of phosphorus, nitrogen, and potassium. He found that total phosphorus dropped to prefertilization figures in 40 days, and that CO_2 was "virtually undetectable."[1]

The phytoplankton showed similar quantitative responses to fertilization in all three studies.[2] There were, however, striking differences in dominant species. In contrast to the increase in Chlorophyta and Chrysophyta in lake 227, Langford (1950) found the most pronounced response to fertilization among the diatoms, chiefly *Tabellaria*, *Fragilaria*, and *Melosira*. Smith (1969) found that *Anabaena*, *Spirogyra*, and *Anacystis* were the major genera responding to fertilization of Crecy Lake.

Implications for Other Lakes and Some Remarks on Watershed Management

Since over 50% of the drainage to the Great Lakes is underlain by Precambrian Shield, including

[1]The increase in "bound CO_2" mentioned by Smith is probably the result of titrating anions other than HCO_3^- and CO_3^-, since the latter tend to be unmeasurably low in Shield waters.

[2]Our data from lake 227 in 1970, however, show phytoplankton standing crops nearly 2× higher than in 1969 (D. W. Schindler et al. unpublished data).

TABLE 2. Phytoplankton identified from tube experiments. A dot (.) indicates presence of a species, and a plus (+) indicates that it was numerous.

	Tube no.											
	1	2	3	4	5	6	7	8	9	10	11	12
Cyanophyta												
Aphanocapsa elachista W. et G.S. West	.	.		.				.				
Aphanocapsa spp.	.	.		.				.				
Aphanothece spp.	.	.		.				.				
Oscillatoria lauterbornii Schmidle					.	.	.		.	.	.	.
Oscillatoria limnetica Lemm.					.	+	+		+	.	.	.
Pelogloea bacillifera Laut.			+									
Chlorophyta												
Chlamydomonas spp.	.	+		.	+	+	+	+			+	+
Phacomyxa sphagnophila Skuja			+									
Tetraedron minimum (A.Br.) Hong	.	.		.	+		.	+	+	+	+	+
Crucigenia tetrapedia W. et G.S. West	.	.	.	.	.	.	.		.	.	.	.
Selenastrum spp.	.	.	.	.	.	.	.		.	.	.	.
Ankistrodesmus falcatus (Corda) Ralfs	+	.	.	.	.	+	.		.	.	.	.
Dictyosphaerium pulchellum Wood						+						
Coccomyxa spp.						+	+					
Staurastrum avicula Breb.	+	+	+	+								
Staurastrum cuspidatum v. *divergens* Nordst			+									
Staurastrum paradoxum v. *parvum* West	+	.		.	+	.		+	+	+	+	+
Spondylosium planum W. et G.S. West			.	.	.				.	+		+
Euglenophyta												
Trachelomonas spp.	.							.				
Phacus caudata Hübner								.				
Chrysophyta												
Chrysophyceae												
Chromulina spp.	.			.								
Chrysococcus spp.	.	.	.	+	.			.				
Mallomonas caudata Ivanov								.				
Mallomonas elongata Rev.								.				
Diatomeae												
Rhizosolenia longiseta Zach.								.				
Rhizosolenia eriensis Smith								.				
Tabellaria flocculosa (Roth) Kg.	.	.	+	+	.			.	+			
Synedra acus Kütz	.	.	.	+	.			+	.	+	+	.
Synedra nana Meister						.	.					
Pyrrophyta												
Cryptophyceae												
Rhodomonas minuta Skuja	.	.	.	.	.			.	.	.	.	.
Cryptomonas pusilla Bachm.	.	.	.	+	.	.	+	.	.	.	.	.
Cryptomonas obovata Skuja	.	.		.	.	.	.	+	.	.	.	.
Cryptomonas marsonii Skuja			.									
Peridineae												
Gymnodinium sp.	.	.		.								
Glenodinium sp.								.				
Peridinium palustre (Lind.) Lef.	.											

the areas of highest runoff within the basin, it is not unreasonable to expect the Great Lakes to respond to enrichment in the same way as a small Shield lake, i.e., the same nutrients would probably be limiting. This hypothesis is supported by the similarity of phytoplankton (S. K. Holmgren unpublished data), zooplankton (Patalas 1969, 1971, and personal communication), and benthic algal communities (Fox et al. 1969; Stockner 1971, and personal communication) in the Great Lakes and in small Precambrian Shield lakes.

Rates of annual supply of phosphorus and nitrogen to lake 227 (experimental + natural) are similar to those entering the lower Great Lakes and the

south baisn of Lake Winnipeg (Table 3). On a square-meter basis, all three Great Lakes considered receive more phosphorus and nitrogen annually than lake 227 does. However, since the mean depths of the Great Lakes are far deeper, the annual supply per unit volume is lower, with the exception of the grossly polluted west basin of Erie. The annual input to lake 227 is also compressed into roughly one-third of the year, whereas that to the Great Lakes is more uniformly distributed in time.

Vollenweider (1968) predicted that the degree of eutrophication that different loading rates of phosphorus and nitrogen will cause in a lake is a logarithmic function of mean depth ($\bar{z}$), with deeper lakes tolerating higher input of nutrient per unit area, but less per unit volume. The danger levels[3] predicted on his fig. 17 and 18 (reproduced in modified form as fig. 3.3.1 and 5.1.1 of vol. 2 and 3, respectively, of Anon. 1969) are given in Table 3 for all four lakes. Obviously lake 227, Lake Erie, and the south basin of Lake Winnipeg exceed the predicted danger limit by several times, and must be regarded as eutrophic or in serious danger of becoming so. Lake Ontario's nutrient income is approaching the dangerous limit.

[3]Vollenweider's (1968) "admissible" and "dangerous" limits may be regarded roughly as the dividing lines between oligotrophic and mesotrophic, and mesotrophic and eutrophic lakes, respectively.

Hutchinson (1941, 1944, 1957 p. 727, 1967 p. 311) has argued that phosphorus is the element most likely to limit phytoplankton in lakes, since it is lower in proportion to other nutrient elements in most natural sources of nutrient supply (both natural waters and geological substrates) than in healthy phytoplankton. In this study we have shown (by lake enrichments coupled with tube experiments described above, as well as with bottle bioassays, D. W. Schindler et al. unpublished data) that phosphorus remains limiting even when its rate of supply is greatly increased in relation to other ions (N:P by weight in our additions = 15:1, close to the ratio found in natural plankton). The high ratio phosphorus uptake: phosphorus content of phytoplankton (Rigler 1964), compared with similar ratios for nitrogen (Procházková et al. 1970; Fitzgerald 1968) and carbon (Schindler and Holmgren 1971), may indicate that the ratio of phosphorus to other elements in phytoplankton must not only equal, but considerably exceed that in natural sources before phosphorus cases to be the most limiting nutrient.

Two factors must be kept in mind when considering the ratio of ions in nutrient supplies. (1) The *amount* of phytoplankton produced will generally be related to the quantity of the most limiting nutrient supplied, and is unrelated to ionic ratios in the supply (Chu 1942). The ionic ratio, therefore, does not determine how much phytoplankton will be produced, but indicates which ion is likely to be

TABLE 3. Levels of total phosphorus and total nitrogen supplied to lake 227 (ELA), to Lake Erie and Lake Ontario, to the south basin of Lake Winnipeg, and to a natural Shield lake.

		P		N				Ratio of load to Vollenweider's (1968) dangerous limits		
	$\bar{z}$, *m*	g/m³ per year	g/m² per year	g/m³ per year	g/m² per year	N/P in supply	Water renewal time, *years*	P	N	Source of data
L. 227	4.4	0.077	0.34	1.14	5.0	15:1	4.2	2.8	2.8	Present study; Brunskill and Schindler 1971
L. Erie	18	0.06	1.1	0.38	6.8	5.5:1	2.5	3.9	1.6	Anon. 1969; Vollenweider 1968
L. Erie, west basin only	6.7	1.05	7	6.7	45	6.4:1	–	43.8	18.8	Anon. 1969; Vollenweider 1968
L. Ontario	84	0.009	0.65	0.12	8.3	12.8:1	6.6	0.9	1.3	Anon. 1969; Vollenweider 1968
L. Winnipeg, south basin	8.4	0.13	1.1[a]	0.88	7.4[a]	6.7:1	–	6.1	2.7	G. J. Brunskill unpublished data;
Clear L., Ont.	12.5	0.003	0.04	Insufficient data		–	18	0.2	–	Schindler and Nighswander 1970

[a]Loading from the Red River only. Estimates including other inflows may be 10–20% higher.

limiting. (2) The possibility that factors other than nutrients limit algal production in some cases must not be overlooked. For example, where light attenuation is extreme (whether due to plankton, tripton, or water color), light rather than any nutrient may be the primary limiting factor.

Other calculations may be performed on data given by the International Joint Commission (Anon. 1969). Using their population figures and input figures for lakes Erie and Ontario, one finds that the phosphorus contribution is 2.04 kg P/capita year to Lake Ontario, and 2.56 kg P/capita year to Lake Erie. Judging by the figures given by Vollenweider (1968), human excreta supplies only 20–25% of this. Assuming that these figures represent good estimates for a fully developed agricultural and industrial society, one concludes that very few inhabitants in the watershed would be required to eutrophy the average small lake. For example, the entire phosphorus input to Lake 227 in this study represents a mere 7–9 capita years, if this were introduced directly to the lake.

Obviously when it is desired to maintain Shield lakes in an oligotrophic state, man's activities must be severely restricted, to ensure that phosphorus, nitrogen, and other nutrients entering lakes directly or via their tributary streams and aquifers are kept below critical levels. For any lake the limits for enrichment will depend upon size and depth of the lake, and upon the natural nutrient input, which is in turn dependent upon the size, shape, vegetation, and geochemical composition of the lake drainage and the amount and distribution of precipitation. The type of activities must also be accounted for in planning watershed development; for example, burning or other means of defoliation may be expected to increase runoff of nutrients from watersheds into nearby water (Likens et al. 1970; Rodhe 1964; Wilde 1958; Ahlgren and Ahlgren 1960). The type and location of any sewage-disposal facilities will also be important. Fertilization of trees, lawns, or crops, and keeping of pets or livestock in the drainage basin must be taken into account. In some cases, contamination of surface or groundwater can be expected to supply nutrients to lakes miles away (Lee et al. 1966). When nutrients are introduced to bays or other areas of lakes that have only limited water circulation with main bodies of water, management schemes for these bays should be adjusted to dimensions of the bays, and not to the lake proper.

Even though data currently available are insufficient to construct a detailed management scheme based upon the above-mentioned factors, the rate of deterioration of natural waters is so rapid that some steps must be taken to curb eutrophication. We suggest that for the Canadian Shield, a calculation as described below could be used as a prelimirnay basis for lake management practice.

Vollenweider (1968 fig. 17 and 18; or see Anon. 1969 vol. 2 fig. 3.3.1 or vol. 3 fig. 5.1.1) estimates limits for total nutrient input, based upon mean depth, as mentioned previously. Persons unable to obtain Vollenweider's figure may approximate these limits mathematically:

$$\log_{10}P_A = 0.60 \log_{10}\bar{z} + 1.40 \quad (3)$$
$$\log_{10}P_D = 0.60 \log_{10}\bar{z} + 1.70 \quad (4)$$
$$\log_{10}N_A = 0.60 \log_{10}\bar{z} + 2.57 \quad (5)$$
$$\log_{10}N_D = 0.60 \log_{10}\bar{z} + 2.87, \quad (6)$$

where P_A and P_D are "admissible" and "dangerous" rates of supply for phosphorus, and N_A and N_D are "admissible" and "dangerous" rates for nitrogen (all in mg/m^2 per year).

Nutrients entering lakes from natural sources may be calculated from equations 1 and 2 given earlier in this paper. Results should be divided by A_o to give units of mg/m^2, compatible with equations 3–6. Subtracting these results from equations 3–6 gives the amount of nutrient that could be permitted from cultural sources (sewage, detergents, fertilizers, deforestation, soil tillage, etc.).

As an example, a calculation for phosphorus input to lake 227 was made using the following data: $A_d = 49 \times 10^4$ m^2; $A_o = 5 \times 10^4$ m^2; $z = 4.4$ m; $p = 0.615$ m.

From equation 4, the "dangerous" limit for phosphorus input would be roughly 120 mg/m^2 per year. The "admissible" limit (equation 3) would be about 60 mg/m^2 per year. Natural sources, as calculated earlier, supply about 20 mg/m^2 per year, so that phosphorus from cultural sources should not exceed 40–100 mg/m^2 per year, depending upon the level of productivity and water clarity that is desired. For lake 227, this would be equivalent to slightly over 1 capita-year for the "admissible" level or 2 capita-years for the "dangerous" level, based upon the previously mentioned average figure of 2.56 kg P/capita-year for the Lake Erie watershed (assuming all phosphorus is supplied to the lake water).

Several limitations of the above model should be clear to the reader. First, Vollenweider's limits have not been extensively tested, and although the characteristics (for example, transparency, algal standing crop) of all lakes that have been examined appear to fit the appropriate areas of Vollenweider's figure, the limits represent no more than the best intuitive estimate of a very experienced scientist.

Second, few data are available on nutrient input to natural lakes. For many situations it will be im-

possible to measure the quantity of precipitation, runoff, and groundwater (if important), as well as their nutrient concentrations. Based upon data for rainfall and runoff for eastern and northwestern Ontario (Schindler and Nighswander 1970; Armstrong and Schindler 1971; Barica and Armstrong 1971; Freshwater Institute unpublished data) an approximate natural input of 20–40 mg P per year for each square meter of watershed ($A_d + A_o$) would appear to be a realistic estimate for Shield lakes.

All of the lakes observed by Vollenweider were large, and more data are needed for ponds and small lakes, although lake 227 and Clear Lake (Schindler and Nighswander 1970) fit the predicted pattern well. The phosphorus supplied per capita year to Lake Erie is probably a maximum value for a drainage basin where agriculture and industry are highly developed. Therefore, results of the calculation outlined above are probably on the conservative side. In a management scheme based upon calculations as crude and untested as ours, more data would be desirable. However, restoration of lakes and their watersheds is much more costly and difficult than a "quota" system, based upon nutrient loads and perhaps other characteristics of lakes. These loads could be slowly revised upward over a period of years as calculations and data improve, and as techniques for eliminating cultural sources of phosphorus are developed. The incorporation of a "safety factor" of approximately two to three times the "useful working load," a familiar concept to engineers, may prove useful for environmental management, where the parameters needed for accurate predictions are difficult to measure.

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Distribution of Radioactive and Stable Zinc in an Experimental Marine Ecosystem

FORD A. CROSS, JAMES N. WILLIS, AND JOHN P. BAPTIST

National Marine Fisheries Service
Atlantic Coastal Fisheries Center
Beaufort, North Carolina 28516, USA

CROSS, F. A., J. N. WILLIS, AND J. P. BAPTIST. 1971. Distribution of radioactive and stable zinc in an experimental marine ecosystem. J. Fish. Res. Bd. Canada 28: 1783–1788.

The distribution of radioactive and stable Zn in an experimental marine ecosystem is described. We added 1 mCi of ^{65}Zn to 2000 liters of sea water and a mixed community of planktonic and benthic algae in a fiberglass tank, after which the ecosystem was left undisturbed for 9 months. We then measured the ^{65}Zn specific activities for (1) particulate Zn (0.45 μ filterable), (2) extractable Zn (from water passed through a 0.45-μ filter and then treated with dithizone), (3) total dissolved Zn (from samples passed through a 0.45-μ filter and treated with acid prior to extraction with dithizone), and (4) dialyzable Zn. Zinc-65 specific activities were not significantly different among the extractable, dialyzable, or acid-treated samples of the water. Further, absolute concentrations of ^{65}Zn and total Zn were no higher in the acid-treated samples than in the extracted or dialyzed samples, which indicates that nearly all of the soluble Zn in the water was dithizone-extractable and dialyzable. Zinc-65 specific activities were significantly less, however, in the particulate material than in the water. This difference indicates that equilibrium of Zn between particulate material and water had not been reached after 9 months. Three months later (December 1969) ^{65}Zn specific activities were determined again for particulate material and total dissolved Zn. The mean specific activity of ^{65}Zn in the particulate samples showed a small but significant increase, but it was still less than in the water. Concentration factors in the particulate matter were 980 for ^{65}Zn and 1400 for total Zn. Samples of benthic algae taken in March 1970 had a mean specific activity of ^{65}Zn similar to the mean ^{65}Zn specific activity of water samples taken 3 months earlier.

CROSS, F. A., J. N. WILLIS, AND J. P. BAPTIST. 1971. Distribution of radioactive and stable zinc in an experimental marine ecosystem. J. Fish. Res. Bd. Canada 28: 1783–1788.

Les auteurs décrivent la répartition du Zn radioactif et stable dans un écosystème halyeutique expérimental. Ils ont ajouté 1 mCi de ^{65} Zn à 2000 litres d'eau de mer contenant une communauté mixte d'algues planctoniques et benthiques en vivier de fibre de verre et laissé tel quel pendant 9 mois. Ils ont ensuite mesuré l'activité spécifique du ^{65}Zn en vue de déterminer (1) le Zn particulaire (filtrable à 0.45 μ), (2) le Zn extractible (à partir de l'eau passée à travers un filtre de 0.45 μ et traitée au dithizone), (3) le Zn dissous total (à partir d'échantillons passés à travers un filtre de 0.45 μ et traités à l'acide avant leur extraction au dithizone), et (4) le Zn dialysable. Aucune différence appréciable n'a été décelée dans l'activité spécifique du ^{65}Zn des échantillons d'eau extractibles, dialysables et traités à l'acide. De plus, la teneur absolue en ^{65}Zn et en Zn total n'était pas plus élevée dans les échantillons traités à l'acide que dans ceux soumis à l'extraction ou à la dialyse. Presque tout le Zn soluble semble donc extractible ou dialysable au dithizone. Cependant, l'activité spécifique du ^{65}Zn était plus faible dans le matériel particulaire que dans l'eau. Cette différence significative prouve qu'après 9 mois, il n'y a pas encore équilibre entre le Zn du matériel particulaire et celui de l'eau. Trois mois après, soit en décembre 1969, l'activité spécifique du ^{65}Zn fut mesurée de nouveau en vue de déterminer le Zn du matériel particulaire et le Zn dissous total. L'activité spécifique moyenne du ^{65}Zn des échantillons particulaires accusa une augmentation légère, mais significative, tout en demeurant inférieure à celle de l'eau. Les coefficients de concentration dans la matière particulaire étaient de 980 pour le ^{65}Zn et 1400 pour le Zn total. L'activité spécifique du ^{65}Zn des échantillons d'algues benthiques prélevés en mars 1970 était la même que celle d'échantillons d'eau prélevés 3 mois plus tôt.

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ALTHOUGH many laboratory experiments have been conducted in recent years to determine uptake and elimination rates and concentration factors of ^{65}Zn in aquatic organisms, few comparisons exist between the distributional patterns of this radionuclide and its stable isotopes in experimental systems. Yet, results of several workers indicate that the concentrations of stable Zn used in laboratory experiments significantly affect uptake and elimination rates of ^{65}Zn in marine organisms (Bryan 1966; Hiyama and Shimizu 1964) and that the initial rate of uptake for ^{65}Zn may be different from the uptake rate for stable Zn (Bernhard and Zattera 1969). In addition, maximum concentration factors can be obtained only when the specific activity of a radionuclide in the experimental organisms equals the specific activity in the water if both forms of the element behave chemically the same. Laboratory experiments designed to determine concentration factors of ^{65}Zn in aquatic organisms, therefore, must be of sufficient duration to allow equilibration between radioactive and stable Zn in the experimental system. The discrepancies between concentration factors of ^{65}Zn in marine organisms determined in short-term laboratory experiments and concentration factors of stable Zn determined in the field are summarized by Polikarpov (1966). Bryan (1966) demonstrated that crabs (*Carcinus maenas*) that had accumulated ^{65}Zn from sea water for 22 days had substantially lower concentration factors for ^{65}Zn than for stable Zn in all tissues and organs sampled. In the present experiment we introduced 1 mCi of ^{65}Zn into an experimental marine ecosystem and then examined the distribution of radioactive and stable Zn in the plankton, attached algae, and several water fractions over a period of 15 months.

Materials and Methods

THE EXPERIMENTAL ECOSYSTEM

In April 1968, 2000 liters of cotton-filtered sea water (30‰ salinity) was added to a fiberglass tank along with culture medium (Rice 1953) and a mixed culture of phytoplankton (*Chlamydomonas* sp., *Chlorella* sp., *Dunaliella* sp., *Carteria* sp., and *Nitzschia closterium*). Eight fluorescent lights were suspended above the tank and set to supply light automatically 12 hr/day. The tank was not disturbed for 9 months, except for weekly additions of deionized water to compensate for evaporation. Ambient temperature of the laboratory in which the tank was located was maintained at 20 ± 4 C. By January 1969, a "natural" population of attached algae had developed in the tank. These algae consisted mainly of *Spirulina* sp. and *Lyngbya* sp., as well as many diatoms and green and red algae. During this time, no planktonic blooms had occurred, and the water in the tank remained relatively clear.

On January 17, 1969, 1 mCi of high specific activity ^{65}Zn (1.17 mCi/mg) in dilute HCl was added to the tank and a moderate supply of air introduced into the bottom of the tank to provide circulation. Since previous studies showed that dye introduced into the tank was completely dispersed within 30 min, we assumed that the ^{65}Zn would be distributed in a similar manner.

After the introduction of ^{65}Zn, the stable and radioactive Zn were left to equilibrate in the ecosystem. During the month of April 1969, a planktonic bloom began in the tank. This bloom was sampled on two occasions (September and December 1969) to compare the ^{65}Zn specific activity of the particulate fraction with the ^{65}Zn specific activities of several water fractions and to calculate a concentration factor of ^{65}Zn for the particulate fractions.

SAMPLING PROCEDURES

In September 1969, ^{65}Zn specific activities were determined for (1) particulate Zn (0.45 μ filterable), (2) extractable Zn (from water passed through a 0.45-μ filter, then treated with dithizone), (3) total dissolved Zn (from samples passed through a 0.45-μ filter and treated with acid prior to extraction with dithizone), and (4) dialyzable Zn. This sampling procedure was carried out in the following manner.

Ten 1-liter samples of water were filtered separately through a 0.45-μ Millipore filter (142-mm diam) in a Teflon-coated filter holder with a polyethylene fritted disc. Two 200-ml aliquots of the filtrate were taken from each sample for measurements of ^{65}Zn specific activities for extractable and total dissolved Zn. In addition, 10 dialysis bags, each holding 300 ml of deionized water, were suspended in the tank for 6 days after which ^{65}Zn specific activity measurements were made on their contents.

Specific activities of ^{65}Zn in particulate material and for total dissolved Zn were measured again in December 1969. This time, however, 3.5 liters of sea water were used for each of 10 samples. Immediately after filtering each sample, the filtrate was refiltered and this second filter was used as a blank. After each sample was filtered a second time, a 200-ml aliquot of the filtrate was then taken for ^{65}Zn specific activity measurements of total dissolved Zn. In addition, 10 samples of attached algae were taken from the sides of the tank on March 7, 1970, for ^{65}Zn specific activity measurements.

ANALYTICAL PROCEDURES

Particulate Zn — The filter papers with the particulate samples collected in September 1969, along with 10 filter paper blanks, and the samples of attached algae collected in March 1970 were dried at 100 C for 48 hr and then dry-ashed in a muffle furnace at 400 C. The filter papers and blanks from the December collections were dried as described above and then digested with successive additions of concentrated HNO_3, $HClO_4$, and HCl.

All measurements for ^{65}Zn were made on a 4 × 4-inch NaI(Tl) crystal coupled to a 512-channel pulse height analyzer. The resulting data were corrected for

decay, instrument efficiency, geometry, and background radioactivity. Total Zn analyses were determined by the dithizone extraction method (Duke et al. 1966), except that atomic absorption spectrophotometry was used for total Zn measurements in the attached algae.

Dissolved Zn — Measurements for ^{65}Zn specific activity of the extractable fraction of 0.45-μ filtered water were made after extraction with dithizone. Measurements of ^{65}Zn specific activities of total dissolved Zn were made on samples of 0.45-μ filtered water that were treated with successive additions of concentrated HNO_3, $HClO_4$, and HCl (Cross et al. 1970) prior to dithizone extractions. The water from the dialysis bags was also treated as described above, subsequent to ^{65}Zn specific activity measurements. Zinc-65 was analyzed in the same manner as with the particulate samples.

Results

Comparisons of ^{65}Zn Specific Activities

Mean values of ^{65}Zn specific activities were similar in the acid-treated water samples, dithizone-extracted, and dialyzed samples (Table 1), as well as the acid-treated water samples taken in December (Table 2). Analyses using *t*-tests showed that none of these means differed significantly ($P < 0.5$). Further, absolute concentrations of ^{65}Zn and total Zn were not higher in the acid-treated samples than in the extracted or dialyzed samples, which indicated that nearly all of the soluble Zn in the water was dithizone-extractable and dialyzable.

The ^{65}Zn specific activities for the particulate samples collected in September and December were significantly less than those obtained for the acid-treated samples ($P < 0.5$). The particulate fraction consisted mainly of *Chlorella* sp., *Nitzschia closterium*, and bacteria, as well as smaller numbers of dinoflagellates. In addition, although the ^{65}Zn specific activities of the acid-treated samples collected in September and December did not differ significantly, the mean concentration of ^{65}Zn decreased from 309 to 192 nCi/liter and the concentration of total Zn decreased from 10.6 to 6.9 µg/liter. During this period the phytoplankton population decreased and the benthic algae present on the bottom and along the sides of the tank grew appreciably. The decrease in the concentrations of both ^{65}Zn and total Zn in the water may have been caused by the accumulation of Zn in the benthic algae. If this were true, then the new growth of benthic algae should have a specific activity of ^{65}Zn similar to the water, because the specific activity did not change significantly when the concentrations of ^{65}Zn and total Zn decreased in the water. Listed in Table 3 are the ^{65}Zn specific activities from 10 samples of attached algae taken in March 1970. Because no samples of water were taken along with the samples of benthic algae, we were not able to compare the ^{65}Zn specific activities of the benthic algae and water statistically. The mean specific activity of the benthic algae samples, however, was very similar to the ^{65}Zn specific activity of the water in the samples of September and December 1969.

Concentration factors for ^{65}Zn and total Zn were also calculated for the particulate material collected in December 1969. Wet weight of particulate material was estimated by dividing the dry weight of each sample by 0.2. Mean concentration factors were 980 for ^{65}Zn and 1400 for total Zn (Table 4). These concentration factors were based on particulate material stopped by a 0.45-µ filter, and therefore represent the average concentration factor for a mixed phytoplankton community along with bacteria and detritus under conditions of the experiment.

Discussion

The differences in ^{65}Zn specific activities between the particulate material and water may have been due to (1) an isotopic effect between ^{65}Zn and stable Zn, (2) a relatively large pool of nonexchangeable Zn in the particulate material, (3) a very slow exchange of Zn between particulate material and water, or (4) the presence of a nonexchangeable "pool" of organically complexed Zn that was available to the phytoplankton but did not exchange readily with dissolved inorganic Zn including ^{65}Zn.

The first explanation can be ruled out due to the similarity in mass between ^{65}Zn and the most abundant stable Zn isotopes (^{64}Zn and ^{66}Zn), which comprise about 76.7% of all Zn in nature.

The second explanation might provide a feasible answer to these differences if a relatively large amount of nonliving matter was present in the tank. This material could contain a substantial quantity of stable Zn that would decrease the ^{65}Zn specific activity of the filterable material. This was not the case, however, as the particulate material consisted mainly of *Chlorella* sp., *Nitzschia closterium*, and bacteria, and only a relatively minor amount of inorganic detritus was present in the water.

Possibly the exchange of Zn between the particulate material and water was relatively slow under the conditions of the experiment and equilibration of Zn had not yet occurred. The small but significant increase in the mean ^{65}Zn specific activity of the particulate fraction from 15 in September 1969 to 21 in December 1969 also indicates a slow rate of exchange between the particulate fraction and water.

The possibility that coastal marine organisms may utilize Zn that is chelated with dissolved organic compounds is discussed by N. H. Cutshall (unpublished

TABLE 1. Concentrations of ^{65}Zn, total Zn, and specific activity of ^{65}Zn for total dissolved Zn (acid-treated), extractable Zn, dialyzable Zn, and particulate Zn for September 1969. All ^{65}Zn measurements were corrected for decay to September 10, 1969.

	Acid-treated Zn			Extractable Zn			Dialyzable Zn			Particulate Zn		
Sample	^{65}Zn	Total Zn	Specific activity	^{65}Zn	Total Zn	Specific activity	^{65}Zn	Total Zn	Specific activity	^{65}Zn	Total Zn	Specific activity
No.	*nCi/liter*	*μg/liter*	*nCi/liter μg/liter*	*nCi/liter*	*μg/liter*	*nCi/liter μg/liter*	*nCi/liter*	*μg/liter*	*nCi/liter μg/liter*	*nCi/sample*	*μg/sample*	*nCi/sample μg/sample*
1	302	8.2	37	320	12.0	27	315	6.6	48	63.3	3.3	20
2	301	10.3	29	244	10.0	24	294	7.1	41	61.0	4.4	14
3	268	9.0	30	230	8.7	26	314	8.2	38	68.4	4.7	15
4	326	9.8	33	284	9.9	29	314	11.1	28	40.8	2.4	17
5	317	9.8	32	308	8.7	35	310	10.8	29	42.3	2.4	18
6	313	8.4	37	308	9.4	33	331	11.3	29	38.5	3.4	11
7	298	14.7	20	313	9.0	35	326	14.4	23	44.3	2.4	19
8	327	9.3	35	320	10.8	30	310	8.7	36	50.5	18.3	3
9	319	8.5	38	220	8.3	27	325	9.0	36	37.7	2.4	16
10	314	18.2	17	318	9.3	34	321	9.6	33	45.0	2.7	17
Mean±SE	309±6	10.6±1.0	31±2	287±13	9.6±0.4	30±1	316±3	9.7±0.7	34±2	49.2±4.0	4.6±1.5	15±2

TABLE 2. Concentrations of ^{65}Zn, total Zn, and specific activity of ^{65}Zn for total dissolved Zn (acid-treated) and particulate Zn in the water for December 1969. All ^{65}Zn measurements were corrected for decay to September 10, 1969.

Sample	Acid-treated Zn			Particulate Zn		
	^{65}Zn	Total Zn	Specific activity	^{65}Zn	Total Zn	Specific activity
No.	*nCi/liter*	*μg/liter*	*nCi/liter μg/liter*	*nCi/sample*	*μg/sample*	*nCi/sample μg/sample*
1	215	6.4	34	55.0	2.7	20
2	182	9.5	19	61.1	3.2	19
3	194	7.2	27	59.1	4.4	13
4	206	7.1	29	68.4	2.8	24
5	167	5.8	29	69.9	3.5	20
6	203	6.6	31	41.3	1.3	32
7	172	7.1	24	74.7	4.3	17
8	179	7.6	24	65.1	4.2	16
9	183	6.0	31	82.5	3.5	24
10	217	6.0	37	64.1	2.9	22
Mean±SE	192±6	6.9±0.3	29±2	64.1±3.6	3.3±0.3	21±2

TABLE 3. Concentrations of ^{65}Zn, total Zn, and specific activity of ^{65}Zn in attached algae for March 1970. All ^{65}Zn measurements were corrected for decay to September 10, 1969.

Sample	^{65}Zn	Total Zn.	Specific activity
No.	*nCi/sample*	*μg/sample*	*nCi/sample μg/sample*
1	2980	82.0	36
2	2770	81.6	34
3	1490	50.1	30
4	784	29.8	26
5	2430	63.8	38
6	2920	80.8	36
7	2570	67.6	38
8	1520	41.9	36
9	2510	65.4	38
10	5300	157	34
Mean±SE	2530±384	72.0±11.0	35±1

TABLE 4. Concentration factors for ^{65}Zn and total Zn for the particulate samples taken in December 1969

Sample no.	Concentration factor	
	^{65}Zn	Total Zn
	nCi/g wet wt nCi/ml	*μg/g wet wt μg/ml*
1	710	1200
2	990	1000
3	920	1800
4	840	1000
5	1200	1700
6	540	530
7	1200	1600
8	1000	1600
9	1500	1900
10	850	1400
Mean	980	1400

data). Dying and decomposing cells may have released organic compounds that were readily assimilated by the living cells. If Zn was complexed with these compounds and was not readily exchangeable with dissolved inorganic Zn, equilibration of ^{65}Zn specific activities between the cells and water could not have occurred. The experiment was 9 months old when the ^{65}Zn was added to the tank, and a substantial quantity of biologically available Zn could have been present in the water that did not exchange readily with ^{65}Zn when it was added. Further work is necessary to clarify the processes governing the differences in ^{65}Zn specific activities between the water and particulate material found in this experiment. The results of this work, however, do point out the need to study the behavior of stable elements in addition to their radioisotopes in tracer experiments.

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Interaction Between Paralytic Shellfish Poison and Melanin Obtained from Butter Clam (*Saxidomus giganteus*) and Synthetic Melanin[1]

R. J. PRICE AND J. S. LEE

Department of Food Science and Technology
Oregon State University, Corvallis, Oreg. 97331, USA

PRICE, R. J., AND J. S. LEE. 1971. Interaction between paralytic shellfish poison and melanin obtained from butter clam (*Saxidomus giganteus*) and synthetic melanin. J. Fish. Res. Bd. Canada 28: 1789–1792.

The interaction between paralytic shellfish poison (PSP) and melanin from butter clam (*Saxidomus giganteus*) and synthetic melanin followed a pattern similar to that reported between PSP and a cation exchanger. The close relation between the anatomical distribution of melanin in butter clams and the distribution of PSP in toxic butter clams suggests that melanin may play an important role in the retention of PSP by these clams.

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Le toxique paralysant des mollusques (PSP) à avec la mélanine de la coque de Washington (*Saxidomus giganteus*) et la mélanine synthétique une action réciproque semblable à celle du PSP avec un échangeur de cations. Le fait que la mélanine est intimement associée au PSP dans les tissus de la coque donne à entendre que ce pigment joue un rôle important dans la rétention du PSP par la coque de Washington.

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FILTER-feeding mollusks such as clams and mussels may accumulate paralytic shellfish poison (PSP) without harm to themselves by ingesting toxic dinoflagellates (Ray 1970). In most shellfish species the PSP is released within a few weeks following a toxic dinoflagellate bloom, but in butter clams (*Saxidomus giganteus*) the poison apparently moves from the hepatopancreas to the siphon, where it is bound and may be retained by the clam for at least 2 years (Quayle 1969; Schantz 1969). From 75 to 88% of PSP content of toxic butter clams may be found in the siphon, gill, and mantle, with the siphons alone containing from 50 to 75% of the PSP (Quayle 1969; Ray 1970). PSP is not uniformally distributed in siphons, but is concentrated in the black tips, and the concentration decreases from the distal to the proximal portion of the siphon (Quayle 1969).

The PSP distribution pattern in butter clam siphons appears to correspond to the areas of melanin pigmentation; the present investigation was undertaken to examine the nature of PSP–melanin interaction and to assess the role of melanin in retention of PSP in butter clams.

Materials and Methods

BUTTER CLAM MELANIN

Microscopic examination of a thin section of a butter clam siphon revealed that the melanin granules are concentrated in the epithelial layers; further studies demonstrated that they could be removed readily by sonication. Melanin from clam siphons was isolated as follows: approximately 57 g of pooled siphons were placed in 150 ml of $\frac{1}{10}$ dilution of McIlvaines standard buffer (Lange 1967), at *p*H 6.0, and held in an ice bath. Unless otherwise noted, the buffer used throughout this investigation was prepared as follows: buffers from *p*H 3.0 to 8.0 were a $\frac{1}{10}$-dilution of McIlvaines standard buffer (Lange 1967) prepared from 0.01 M citric acid and 0.02 M Na_2HPO_4. Buffers at *p*H 2.0–2.5 were prepared from 0.01 M citric acid and adjusted with 1 N HCl. The siphon suspension was subjected to sonication for 3 min using a Biosonic sonic oscillator (Brownwill BP 111 with standard probe). This effectively separated the melanin from the

[1]Technical Paper No. 3103, Oregon Agricultural Experiment Station. Based on a thesis to be submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the Department of Food Science and Technology, Oregon State University, Corvallis, Oreg.

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epithelial cells of the siphons. The sonicated suspension was filtered through a layer of cheese cloth to remove remaining siphons. Cellular debris was removed from the filtrate by centrifugation at 200 × g for 10 min. Melanin granules were collected by centrifugation at 20,000 × g for 20 min, washed twice with buffer at *p*H 6.0, and suspended in 300 ml of 0.05 M phosphate buffer at *p*H 7.0. Protein was removed from the suspension by treatment with papain (EC 3.4.4.10; Sigma Chemical Company) at 25 C for 48 hr, according to the method of Arnon (1970). The granular suspension of melanin was washed by centrifugation with 0.1 N HCl until the supernatant gave a negative ninhydrin reaction. Centrifugation of the washed melanin yielded a blackish-brown pellet weighing approximately 500 mg and essentially free of protein. The melanin preparation was suspended in 100 ml of 0.1 N HCl to contain 1.5 mg melanin by dry weight/ml and stored at 4 C.

Synthetic Melanin

Synthetic melanin was prepared from L-β-3,4-dihydroxyphenylalanine (L-DOPA, Sigma Chemical Company) with mushroom tyrosinase (*o*-Diphenol: O_2 oxidoreductase, EC 1.10.3.1, Sigma Chemical Company). Five milligrams of L-DOPA and 500 units of tyrosinase in 20 ml of buffer at *p*H 6.0 were incubated at 25 C with shaking for 24 hr. Under these conditions, 5 mg of L-DOPA yielded 2.8–3.0 mg of pure melanin.

Paralytic Shellfish Poison

Purified PSP was dissolved in 0.001 M HCl, and stored at 4 C. A solution containing 100 μg of PSP/ml was used as a standard.

PSP solutions were assayed with the Folin and Ciocalteau phenol reagent (Fisher Chemical Company). Two-milliliter samples were mixed with 0.25 ml of phenol reagent, 0.5 ml of saturated aqueous sodium carbonate, and 0.5 ml of distilled water. The tubes were incubated at room temperature for 1 hr, and absorbance at 660 nm was measured with a Bausch and Lomb Spectronic 20 spectrophotometer. Under these conditions, concentrations of PSP from 1 to 15 μg/ml could be measured accurately (unpublished data). Although the Folin–Ciocalteau reagent is relatively nonspecific, it was used in our experiments since the melanin preparation was freed from interfering compounds by repeated buffer washes, and melanin is essentially insoluble in aqueous solution under neutral or acidic conditions (White 1958). The Folin–Ciocalteau procedure is simpler than the modified Jaffe test developed by McFarren et al. (1959), which requires solvent extractions prior to absorbance readings.

PSP–Melanin Binding

Melanin suspensions in 0.1 N HCl were washed by centrifugation three times with buffer prior to binding experiments. The binding of PSP to synthetic and clam melanin was measured by adding 26 μg of PSP in 0.001 M HCl to melanin suspended in 2.6 ml of buffer and incubating at 25 C in a water bath shaker. After 1 hr incubation, melanin was separated by centrifugation at 20,000 × g for 20 min, and the supernatant fluid assayed for PSP. The release of bound PSP was measured by washing the melanin–PSP complex once with 3 ml of buffer at *p*H 6.0 and then suspending the melanin in 3-ml portions of buffer from *p*H 3.0 to 6.0 and incubating for 15 min. The melanin was separated by centrifugation, the supernatant fluid assayed for PSP, and the melanin suspended in another 3 ml of buffer. This procedure was repeated four times.

Preliminary experiments binding 26 μg of PSP to 2.8 mg of synthetic melanin for 1, 2, 4, 8, and 24 hr indicated that equilibrium was attained within 1 hr and remained constant for at least 24 hr. Therefore, 1-hr incubation periods were used in all binding experiments.

Figure 1 shows the quantitative relation between melanin concentration and the degree of melanin–PSP binding. Three milliliters of the stock melanin solution, containing approximately 4.5 mg of clam melanin, were used in all binding experiments. This amount of clam melanin binds from 63 to 67% (16–17 μg) of PSP at *p*H 6.0.

Results and Discussion

The percent of PSP bound to synthetic and clam melanin in buffer of *p*H 2.0–8.0 is shown in Fig. 2. The degree of binding between PSP and melanin was strongly influenced by *p*H. The binding increased with increasing *p*H from *p*H 2.0 to 5.0. With clam melanin, it reached a maximum of 63% at *p*H 6.0. The binding was more dramatic with synthetic melanin, which bound 100% of the PSP at *p*H 5.0. From *p*H 4.0 through 8.0, the percent of PSP bound to synthetic melanin was near 100.

The difference between synthetic and clam melanin may be due to a difference in the number of effective reactive sites. The synthetic melanin

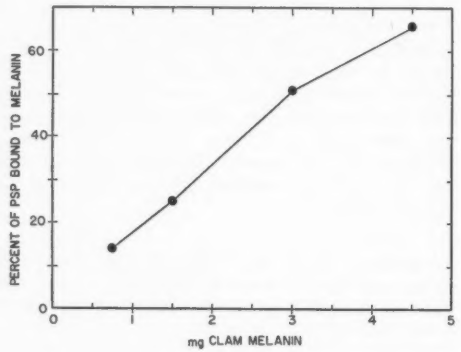

FIG. 1. Effects of melanin concentration on PSP binding in citrate–phosphate buffer at *p*H 6.0.

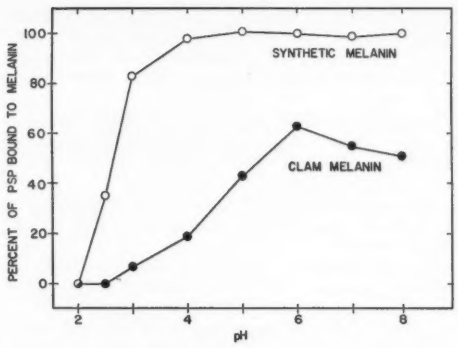

FIG. 2. Effects of *p*H on the binding of PSP to synthetic melanin and clam melanin.

appeared to have a less organized structure, probably permitting greater exposure of reactive sites to PSP. Synthetic melanin made from pure L-DOPA contains indole-5,6-quinone polymer units exclusively (Bruenger et al. 1967), whereas natural melanin could have contained heterogeneous polymer units, which probably have fewer binding sites. Figure 2 indicates that less PSP was bound to clam melanin at *p*H 7.0–8.0 than at *p*H 6.0. This may be due to partial solubilization of the melanin at these *p*H levels. Bruenger et al. (1967) indicated that natural melanin loses some of its granular structure above *p*H 7.0, and they showed a decrease in the binding of 224 Ra^{++} to melanin at *p*H 7.0 and 7.5 when compared with the binding at *p*H 6.0. In our experiments, clam melanin appears to solubilize slightly at *p*H 7–8, as indicated by a slight coloration in buffer washes. Although PSP was shown to decompose slowly in alkaline solution (Schantz et al. 1957), no decomposition was noted in our experiments.

The reversible nature of the PSP–melanin reaction is shown in Fig. 3. The rate of desorption increases with increasing acidity, indicating a competition between hydrogen ions and PSP for binding sites on the melanin.

Natural melanin contains free carboxyl groups and phenolic hydroxyl groups and can function as a cation exchanger (White 1958; Bruenger et al. 1967). The binding of PSP to melanin appears to be such a cation exchange reaction. This explanation is supported by the reversibility of the binding and the increased desorption of PSP from melanin with increasing acidity (Fig. 3), and by the decreased PSP uptake at *p*H 2.0–3.0 (Fig. 2). At low *p*H the weak acidic groups on melanin are in the hydrogen form (Bruenger et al. 1967), thus the uptake of PSP is probably blocked.

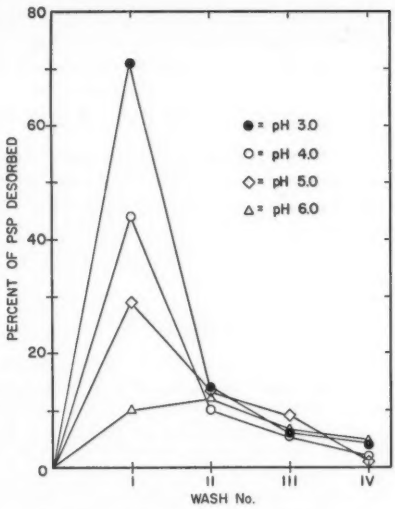

FIG. 3. Effects of *p*H on the desorption of PSP from clam melanin containing 16.5 μg of bound PSP. Melanin was washed with 3-ml portions of citrate-phosphate buffer at 25 C.

The exact mechanism by which butter clams retain PSP is not known. E. J. Schantz, however, has suggested (personal communication) that it is similar to the binding of PSP on a weak cation exchange resin. The present observation on PSP–melanin interaction closely resembles that noted between PSP and whole butter clams, and between PSP and cation exchange resins. McFarren et al. (1958) found that PSP absorbs optimally to Amberlite XE-64 cation exchange resin at *p*H 5.2. At higher *p*H levels, PSP was destroyed by the alkaline conditions or was washed off the column by the buffer. They found that PSP was adsorbed at *p*H 4.5, but a slight decrease in *p*H caused elution of PSP from the column. Extraction of PSP from butter clams is accomplished with dilute acid solutions at *p*H 2–3 (Schantz et al. 1957). This correlates well with the rapid desorption of PSP from melanin at *p*H 3.0 (Fig. 3) and the minimal uptake of PSP by clam melanin at this *p*H (Fig. 2).

The *p*H dependence found for the interaction between PSP and butter clams is similar to that for interactions between protein and metal ions or proteins and charged molecules (Tanford 1961; Keen 1966). Preliminary experiments in our laboratory with PSP and bovine serum albumin did not show binding. In addition, PSP binding to protein does not satisfactorily explain the large variation in toxin retention by different species of mollusks

(Quayle 1969). The melanin content of butter clam appears to vary among individual clams, and the melanin content of the clam may be responsible for the wide range of toxin content observed among different butter clams harvested from the same area (Quayle 1969).

Work is now in progress on further characterization of the nature of the PSP–melanin interaction, on the significance of this interaction in butter clams in relation to other shellfish species, and on the use of the Folin–Ciocalteau phenol reagent in the assay of PSP in toxic butter clams.

Acknowledgments

We are greatly indebted to Dr E. J. Schantz, Fort Detrick, Maryland, for the supply of purified PSP. This investigation was supported in part by National Oceanic and Atmospheric Administration (Administered by U.S. Department of Commerce) Institutional Sea Grant.

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NOTES

Effects of Adaptive and Nonadaptive Feeding of Hydrogenated and Raw Herring Oil on Hearts and Livers of Weanling Rats[1]

P. H. ODENSE AND H. BROCKERHOFF

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ODENSE, P. H., AND H. BROCKERHOFF. 1971. Effects of adaptive and nonadaptive feeding of hydrogenated and raw herring oil on hearts and livers of weanling rats. J. Fish. Res. Bd. Canada 28: 1793–1795.

It has been reported that the feeding of high levels of rapeseed oil to weanling rats leads to the eventual appearance of cardiac lesions. Marine oils have come under suspicion since most of them contain lesser amounts of the long-chain (C_{20} and C_{22}) monounsaturated acids found in rapeseed oil. Weanling rats, male and female, were given diets containing 18% hydrogenated or raw herring oil for 24 days. Two other groups were adapted gradually to these diets during an initial period of 12 days. Control groups were kept on diets containing corn oil and lard. Histological investigations of the hearts failed to produce any evidence of unusual fat deposition or cardiac damage in any of the rats, either control or experimental. The possibility that a more prolonged feeding of the oil could lead to lesions cannot be excluded. Liver weights, when expressed as fractions of the body weights, were slightly, though significantly, higher (13–27%) in all groups fed with raw or hydrogenated herring oil, but histological examination failed to disclose any difference between these livers and those of the control groups. The experiments indicate that herring oil, raw or hydrogenated, when fed to weanling rats for 24 days, produced no untoward histological changes in the heart or liver tissues.

ODENSE, P. H., AND H. BROCKERHOFF. 1971. Effects of adaptive and nonadaptive feeding of hydrogenated and raw herring oil on hearts and livers of weanling rats. J. Fish. Res. Bd. Canada 28: 1793–1795.

Des rapports ont été publiés à l'effet qu'un régime riche en huile de colza produit éventuellement des lésions cardiaques chez les rats en sevrage. Les huiles marines sont devenues suspectes, elles aussi, puisque la plupart contiennent, en quantités moindres, les acides monoinsaturés à longue chaîne (C_{20} et C_{22}) de l'huile de colza. Nous avons soumis pendant 24 jours des rats mâles et femelles en sevrage à des régimes contenant 18% d'huile de hareng hydrogénée ou brute. Deux autres groupes furent graduellement adaptés à ces régimes pendant une période initiale de 12 jours. Les groupes témoins furent soumis à des régimes à base d'huile de maïs et de saindoux. L'examen histologique du cœur n'a fourni aucune évidence de dépôt anormal de graisse ou de lésion cardiaque dans les groupes témoins ou dans les groupes expérimentaux. Nous n'excluons toutefois pas la possibilité qu'un régime prolongé à base d'huile aboutisse à des lésions. Le poids du foie, exprimé en fraction du poids vif, accusait une augmentation légère, mais significative (13–27%), dans tous les groupes nourris à l'huile de hareng brute ou hydrogénée. L'examen histologique du foie n'a cependant révélé aucune différence entre ces groupes et les groupes expérimentaux. Nous en concluons que l'huile de hareng brute ou hydrogénée donnée à des rats en sevrage pendant 24 jours ne produisit aucun changement histologique destructif des tissus cardiaques et hépatiques.

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IT has been reported recently that the feeding of rapeseed oil to weanling rats at levels of 10% or more of their diet leads, after a few days, to the temporary appearance of fat in the heart cells and subsequently to histopathological changes (J. Beare-Rogers et al. unpublished data; Thomasson et al.

[1]A more detailed description of the experiments is available from this laboratory as Fisheries Research Board of Canada Technical Report No. 229.

Printed in Canada (J2087)

1970; Abdellatif and Vles 1970; Roine et al. 1960; Rocquelin and Cluzan 1968; Rocquelin and Potteau 1968). These effects are less marked with hydrogenated rapeseed oil. Since hydrogenated marine oils contain percentages of monounsaturated C_{20} and C_{22} fatty acids comparable with those of hydrogenated rapeseed oil, which are assumed to be the cause of the deposition of fat in the heart, it was considered desirable to investigate if the feeding of hydrogenated (HHO) or even of raw (RHO) herring oil to weanling rats for a period of several weeks would lead to cardiac damage.

A preliminary feeding experiment with HHO as the dietary fat (J. Beare-Rogers unpublished data) had shown fatty deposition in rat hearts, though markedly less than with raw rapeseed oil. The deposition of fat in the hearts of rats on a diet of rapeseed oil or HHO was reversed after the 1st week even when the oil ingestion continued. In consideration of these findings it was decided to include an additional experiment in the present study. Rats were fed diets in which the percentage of HHO or RHO was increased over a period of 12 days. The purpose of the test was twofold: to make the experiment more relevant to human nutrition, where dietary changes during infancy are gradual rather than abrupt, and to take into account the design and results of the rapeseed oil-feeding experiments, which suggested that a sudden flooding of the heart with dietary fat had led to the irreversible damage. Possibly the heart, given time, would activate some latent metabolic system necessary to cope with the long-chain monoenoic acids, and if this adaptation were permitted, massive deposition might never occur.

The animals used were weanling Sprague-Dawley rats, 3 weeks old, supplied by Carworth, New York, N.Y. The fat-free basic diet (Wooley and Sebrell 1945) was supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio. The hydrogenated (i.e., partially hydrogenated) herring oil (HHO) had an iodine value of 76. The fatty acid compositions of HHO and RHO were those expected, and their contents of C_{20} and C_{22} monoenoic acids were in the ranges normal for these fats. A detailed report is in preparation. DL-Alpha tocopherol, at 0.03% (66 I.U. per kg food), was added to all fats.

Diets — Control: 80% "fat-free" diet, 10% lard, 10% corn oil; HHO-diet: 80% "fat-free" diet, 18% HHO, 2% corn oil (to supply essential fatty acids); RHO-diet: 80% "fat-free" diet, 18% RHO, 2% corn oil.

Feeding schedule (all diets were fed ad libitum) — Each group (A–E) was divided into males (A male, etc.), 12–13 animals each, and females (A female, etc.), 12–13 animals each. *Group A* was kept on a control diet for 30 days. *Group B* was adapted to HHO in the course of 12 days. (The concentrations of HHO were increased from day 1 (1%) to day 7 (7%) in steps of 1%, then in steps of 2% to 15% on day 11, then to 18% on day 12. The total fat content was always brought to 20% with the mixture of corn oil and lard.) *Group D* was adapted to RHO in the same manner. *Group C* was kept on the control diet for 6 days, then changed to 18% HHO on the 7th day. *Group E* was kept on the control diet for 6 days, then changed to RHO on the 7th day.

One animal of groups A, C, and E, male and female, was killed with ether on day 13; and one of groups B and C on day 15, for the purpose of studying the initial fat deposition in the heart. All other animals were killed on day 30. The hearts were immediately removed and fixed in 10% buffered saline formalin solution. The livers were also taken and fixed in the same manner after weighing. Portions of all hearts and livers were paraffin-embedded, sectioned, and stained with haematoxylin and eosin. Two sets of frozen sections were cut from five hearts from each experimental group and from all hearts of the 10 rats killed on days 13 and 15. One set was stained with Sudan Black B and the other with Oil Red O as the lipid stain. Both sets were counterstained with haematoxylin. Frozen sections of all the livers were cut and stained with Oil Red O and haematoxylin.

Examination of the H and E stained heart tissue sections failed to reveal any evidence of lesions (Fig. 1*a*, *b*, *c*, *d*). In group A females and group C males, one section in each group showed a possible edaematous tissue patch. In the 220 sections, all other tissue constituents appeared normal. In sections where the membranous portion of the septum between the ventricles was visible, no pathological changes were observed.

The heart sections stained for fat with Oil Red O were a diffuse pinkish colour. No discrete droplets of oil were observed with the light microscope, even under high power. As noted by Ham (1965), electron micrographs of normal cardiac muscle tissue reveal the presence of small fat droplets among the myofibrils. Our results indicate the presence of small amounts of intracellular fat but, regardless of the diet, an absence of any significant fat deposits in the heart.

The frozen liver sections revealed the presence of variable quantities of fat. Each slide was graded as to the variability of the fat deposits in the section, the intensity of staining, the localization of the fat deposits, and the approximate concentrations of small, medium, and large globules of fat (Fig. 1*e*, *f*). The only statistically significant finding was a sex difference in the fat deposits. In each of groups A, B, C, D, and E, the male rats possessed 10–15% higher concentrations of fat globules than the female rats. There were no significant differences between the groups on the basis of their diets.

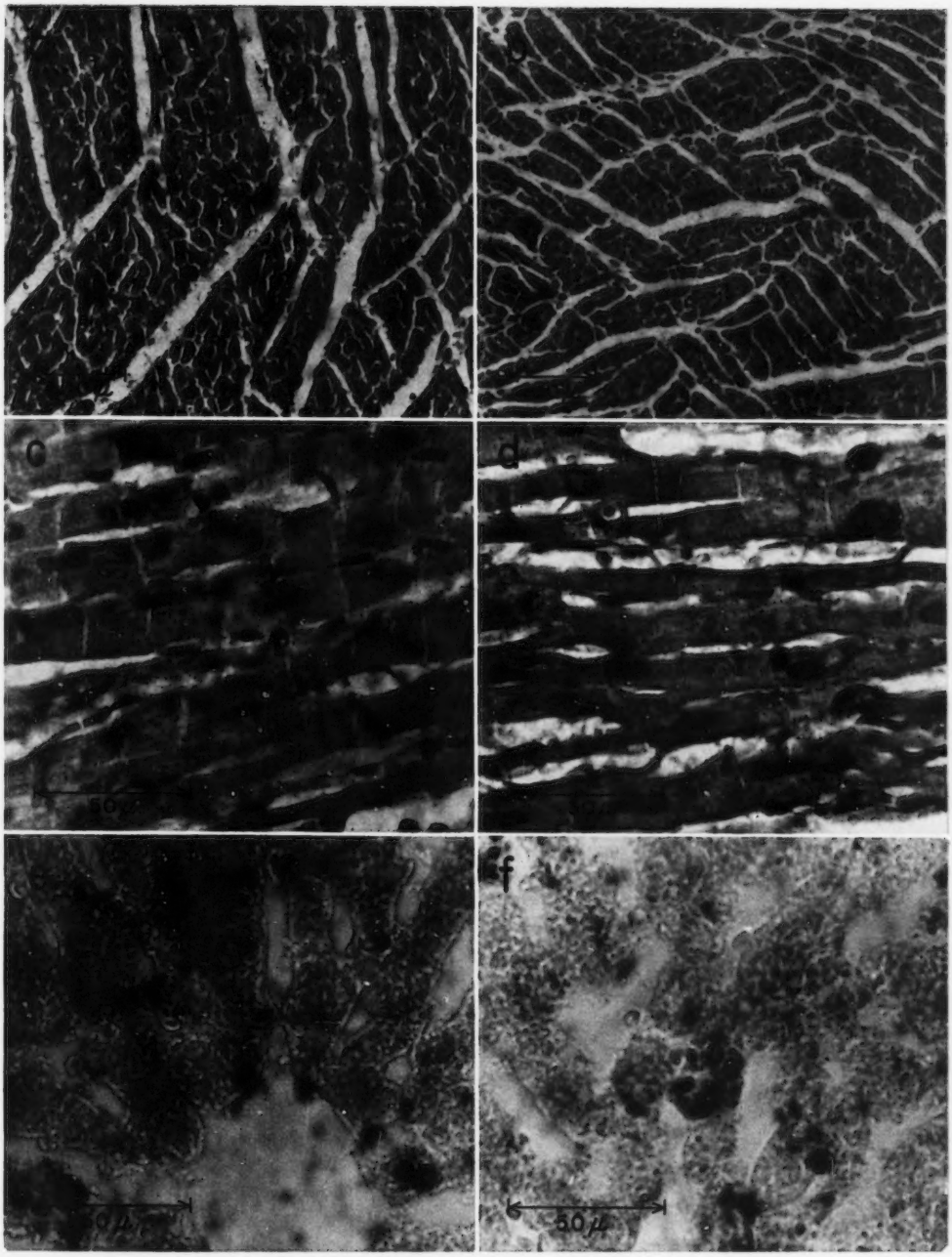

FIG. 1. Photomicrographs of heart and liver sections: (*a*) Cross section of rat heart from group A male, fed control diet. Low power, paraffin-embedded, haematoxylin and eosin stains. (*b*) Cross section of rat heart from group E male fed raw herring oil diet without adaptation period. Low power, haematoxylin and eosin stains. (*c*) Longitudinal section of rat heart from group A male fed control diet. High power, haematoxylin and eosin stains. (*d*) Longitudinal section of rat heart from group E male fed raw herring oil diet without adaptation period. High power, haematoxylin and eosin stains. (*e*) Frozen section of liver from group A male fed control diet. Stained for lipid with Oil Red O and counterstained with haematoxylin. (*f*) Frozen section of liver from group E male, fed raw herring oil diet without adaptation period. Stained with Oil Red O and haematoxylin.

The average weight of the animals in each group was determined at the beginning of the experiment, the weight of the body and liver of each animal at the end. The percentage increase in body weight during the 30 days and the percentage of liver in the body weight was calculated. A statistical analysis (courtesy of W. J. Dyer) indicated a significant difference, especially for the males (at the 1% level) between the body weights in group E (nonadaptive RHO), which were appreciably lower than those of the control group A. It would be rash to conclude that we have here a direct physiological effect of raw herring oil. More likely, the lower weight signified that the rats found the RHO diet distasteful.

The livers, as percent of body weight, were significantly larger (at the 1% level) in all groups fed HHO or RHO, adapted or not. The relative weight increases ranged from 13.4% (group B male) to 27.4% (groups D and E female). If absolute rather than relative weights are compared, then the differences in weight are in all cases reduced, and in some cases disappear (B male, E male). The largest overweight (group D female) is then 20.5% above average. Although the differences in absolute liver weights remain significant in group B male and in all females, they cannot be regarded as pathological, especially when the negative histological findings are considered.

These experiments indicate that herring oil, raw or hydrogenated, fed to weanling rats for 3–4 weeks, produced no untoward histological changes in the heart or liver tissues. Note that the question of whether a more prolonged administration of the oils will eventually lead to cardiac damage has been left unanswered.

Acknowledgments — The authors gratefully acknowledge the advice and assistance provided by Drs R. G. Ackman and W. J. Dyer. They are also indebted to Mr J. Gibson of the Nova Scotia Pathology Institute for technical advice and assistance in the preparation of the liver sections. We are especially obliged to Dr J. Beare-Rogers for communicating her findings to us and for much helpful advice.

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Preliminary Description of an Unusual Gonatid Squid (Cephalopoda: Oegopsida) from the North Pacific

W. GORDON FIELDS AND VERONICA A. GAULEY

Department of Biology
University of Victoria, Victoria, B.C.

FIELDS, W. G., AND V. A. GAULEY. 1971. Preliminary description of an unusual gonatid squid (Cephalopoda: Oegopsida) from the North Pacific. J. Fish. Res. Bd. Canada 28: 1796–1801.

Descriptions are given of two specimens of an unusual type of gonatid squid taken from the North Pacific Ocean. A discussion is made of their relationship to two existing genera, *Gonatus* and *Berryteuthis*, of the family Gonatidae, and a note is included on a North Pacific gonatid larva that may be a related earlier stage of these two specimens. Because of their size and condition, neither a new genus nor a new species is declared; however, on the basis of these records, it is suggested that there is a need for a redefinition of the genera of the family Gonatidae.

FIELDS, W. G., AND V. A. GAULEY. 1971. Preliminary description of an unusual gonatid squid (Cephalopoda: Oegopsida) from the North Pacific. J. Fish. Res. Bd. Canada 28: 1796–1801.

Nous décrivons deux spécimens d'un type peu commun d'encornet de la famille des Gonatidae du Pacifique Nord. Nous discutons leur degré de parenté avec deux genres actuels, *Gonatus* et *Berryteuthis*, de cette famille. Nous mentionnons aussi une larve de gonatidé du Pacifique Nord qui pourrait être un jeune stade apparenté à ces deux spécimens. La taille et la condition des spécimens nous empêchent de créer une espèce ou un genre nouveaux. Par contre, à la suite de ces découvertes, nous croyons que les genres de la famille des Gonatidae doivent être redéfinis.

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DURING a study of several collections of cephalopods on loan to the University of Victoria, two unusual squid belonging to the family Gonatidae were found. Close examination revealed affinities to two genera, *Gonatus* and *Berryteuthis*. Both specimens have a radula with five longitudinal rows of teeth, a *Gonatus* characteristic, and a tentacular club bearing suckers only, a *Berryteuthis* characteristic (Roper et al. 1969).

Since one specimen is an early juvenile and the other an immature female in poor condition, the declaration of a new species or possibly a new genus is deferred until such time as mature specimens in better condition are available. The purpose of the present paper is to contribute information on a hitherto unknown form.

Materials and methods — The larger specimen is held by the University of Victoria in the transpacific *Endeavour* collection (UV-TPE) and the smaller specimen by the National Museum of Canada (NMC). Measurements were made according to Voss (1956), except for club length (Berry 1912).

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Specimens — One immature female, UV-TPE 65 ML 65 mm; North Pacific Ocean, Endeavour station TP-12-10, 45°07′N, 162°53′E, March 30, 1969.

One early juvenile, NMC 48443, ML 31 mm; North Pacific Ocean, Weather station "P", 49°53′N, 145°03′W, September 10, 1969; 0–665 m.

Description — Both specimens possess characteristics of the family Gonatidae, including: tetraserial armature on the arms; the two medial rows of arms I–III comprised of hooks; simple, straight, funnel locking-cartilage; and buccal connectives attaching to the ventral borders of arms IV (Roper et al. 1969).

Specimen UV-TPE 65 — This is a female bearing a few immature eggs in the posterior mantle cavity. It is in poor condition, with damage to the fins, the posterior tip of the mantle, and the flesh around the eyes; it is missing the distal half of right arm III and lacks epidermis on the mantle, fins, aboral side of the arms, and parts of the head.

The thick mantle is cone-shaped, being widest at its anterior margin but not wider than the head (Fig. 1*a*). The anterior mid-dorsal margin projects strongly; however, this appears to be due to contraction of the mantle musculature upon fixation. Ventrally, the margin

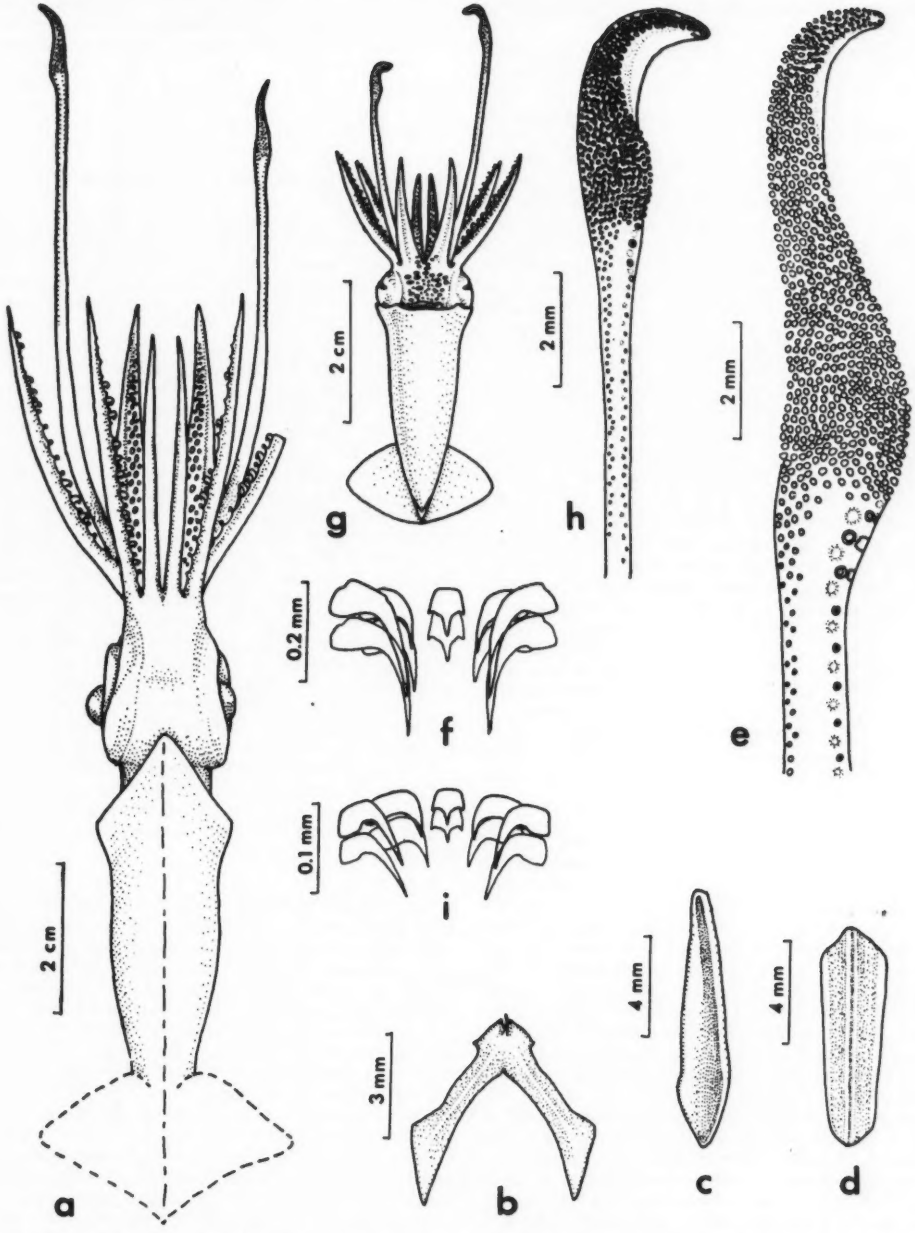

FIG. 1. Drawings of gonatid squid specimens from the North Pacific. Female, UV-TPE 65: (*a*) dorsal view; (*b*) dorsal pad of the funnel organ; (*c*) left funnel locking-cartilage; (*d*) nuchal cartilage; (*e*) right tentacular club; (*f*) radula. Juvenile, NMC 48443: (*g*) ventral view; (*h*) right tentacular club; (*i*) radula.

projects at the mantle components of the funnel locking-cartilage.

The fins are small, less than one-third of the mantle length, and in total breadth a little wider than the mantle. Posteriorly, the fins are subterminal and concave; anteriorly, they are lobed at their point of attachment to the mantle (Fig. 1*a*).

The gladius is slightly visible along the dorsal midline. It was not dissected out.

The funnel tapers evenly from a wide base to a slit-like anterior opening and extends almost level with the center of the eyes. It lies in a deep groove but is attached to the ventral side of the head only by a pair of anterior adductor muscles. The dorsal pad of the funnel organ has the shape of an inverted "V". The middle sections of the "arms" of the V are slender and each bears a narrow ridge extending almost to the apex; the posterior parts of the limbs first widen then taper gradually to a point. Anteriorly, where the limbs join, the organ widens then tapers rapidly to a notched apex and a small, slender, apical papilla (Fig. 1*b*). The ventral pads of the funnel organ are egg-shaped. The funnel locking-cartilage is elongate, widest posteriorly, and bears a simple, straight, median groove (Fig. 1*c*). The mantle component is a prominent straight ridge.

The head is large, wider than the mantle opening, and one-third the length of the mantle (Fig. 1*a*). It is very constricted in the nuchal region. The large, circular eyes form the widest section of the head and occupy its entire lateral surfaces. There is a short, broad, olfactory papilla posterior to each eye. Nuchal folds are not discernible. The nuchal locking-cartilage is long with diverging sides that make the bluntly pointed, anterior margin wider than the rounded posterior margin. A very narrow median groove with prominent lateral ridges extends the length of the cartilage. Both ridges are bordered laterally by a wide, shallow groove (Fig. 1*d*).

The arms are long, ranging from 52 to 61% of the mantle length (Fig. 1*a*). They are stout along most of their length but taper to a fine point. The arm formula is $2 = 3 > 4 > 1$. Arms IV bear a narrow lateral keel. Aboral keels are not evident on arms I, II, and III. All arms bear shallow, trabeculate, protective membranes lateral to the armature. These membranes are deeper on arms I, II, and III than on arms IV. The armature is in four longitudinal series. Arms I, II, and III bear two median rows of very large, acutely pointed hooks; the largest are almost four times the size of the largest arm sucker (Fig. 2*a*, *b*, *c*). Proximally the hooks are preceded by about two partially modified suckers; distally, they are replaced by small suckers for approximately 4 mm at the tip of the arms. The two marginal rows on all arms consist of very small suckers arising from the trabeculae of the protective membranes. Arms IV bear four rows of suckers, the largest of which is larger than any of the suckers on arms I, II, and III. Although there are some hooks and suckers missing, it is estimated that there are 37–41 hooks and 44–55 suckers (excluding the small suckers at the arm tip) on each of arms I, II, and III; and 109 suckers on arms IV. The actual number of hooks and suckers remaining are:

	Left		Right	
Arms	Hooks	Suckers	Hooks	Suckers
I	24	50	28	50
II	29	38	18	32
III	30	37	13	16
IV	–	109	–	109

The arm suckers bear 6–8 short, bluntly pointed teeth (Fig. 2*d*, *e*).

The tentacles and clubs are slender; the right tentacle is shorter than the left, probably because of contraction of the stalk musculature. The club is 15.4% of the mantle length and distinctive in structure for a gonatid of 65 mm mantle length (Fig. 1*e*). Almost the entire oral surface of the club bears numerous, scattered, minute suckers, none of which exhibit any stage of development into the typical *Gonatus* tentacular hooks. Their dentition consists of 5–6 blunt, squarish teeth (Fig. 2*f*). At the tip of the dactylus is a small circlet of 7–8 suckers and, beginning at the tip, is a short, narrow membrane on the dorsal side. Proximally, suckers continue for 10 mm in a single row along the ventral margin of the stalk. The locking zone is comprised of smooth-ringed suckers alternating with rounded knobs (Fig. 2*g*). Three distal suckers, located medially to short transverse ridges, are the largest suckers on the club (Fig. 1*e*). Proximally, on the left tentacle 54 suckers and 66 knobs, and on the right tentacle 50 suckers and 62 knobs continue along the dorsal margin of the stalks for one-half their lengths. Both suckers and knobs consistently decrease in size. These suckers are also smooth-ringed (Fig. 2*h*).

The remaining chromatophores are reddish brown and small. They are scattered over the dorsal and ventral surfaces of the head and the oral surface of the arms.

The buccal membrane is dark reddish brown and bears seven lappets that attach to the dorsal borders of arms I and II and to the ventral borders of arms III and IV.

The radula has five teeth in each transverse row (Fig. 1*f*).

Specimen NMC 48443 — This is a juvenile in fairly good condition (Fig. 1*g*). It has slightly frayed fins and lacks epidermis on the mantle, fins, aboral side of the arms, and parts of the head. Its morphology is the same as that for the larger specimen with the following exceptions. The mantle does not appear to be contracted and there is only a slight projection at the mid-dorsal anterior margin. No olfactory papillae were observed. There is a green and yellow luminescent layer on the ventral surface of the eyes.

The arms range from 42 to 55% of the mantle length and have the formula $3 > 2 = 4 > 1$. The armature is the same as for the larger specimen, except that fewer suckers have been modified into hooks and the existing hooks have not developed extended points (Fig. 2*i*, *j*). On each of arms I, II, and III there are 20–26 hooks and 46–60 suckers. In the median two rows there are 4–6 suckers proximal to the hooks and 10 or more distally. Arms IV have 92 suckers each. The arm suckers bear 4–5 short, blunt teeth (Fig. 2*k*, *l*).

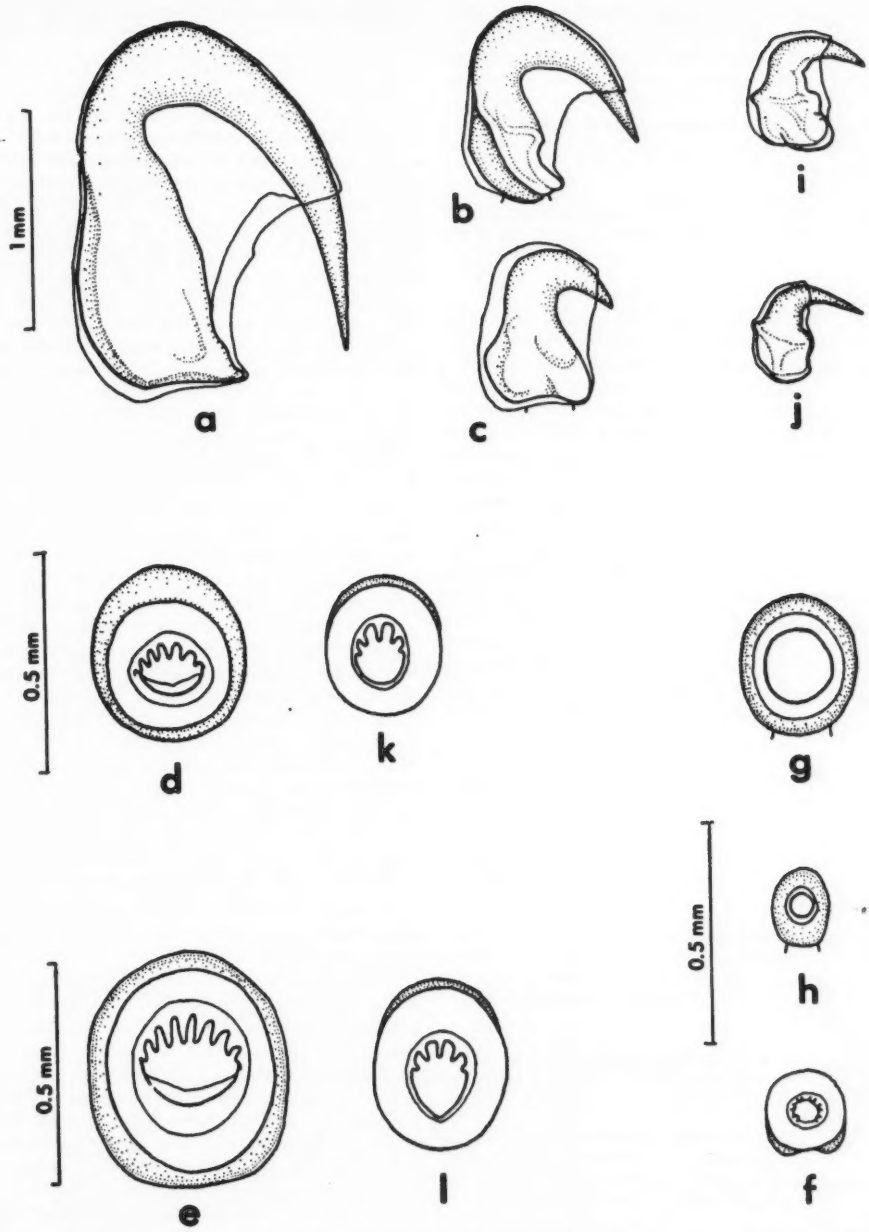

FIG. 2. Armature of gonatid squid specimens. Female, UV-TPE 65: (*a*) largest hook from arm III; (*b*) hook from distal end of arm III; (*c*) hook from proximal end of arm III; (*d*) sucker from arm III; (*e*) sucker from median row of arm IV; (*f*) tentacular club sucker; (*g*) largest sucker of locking zone; (*h*) sucker from dorsal margin of right tentacle stalk. Juvenile, NMC 48443: (*i*) largest hook from arm III; (*j*) hook from distal end of arm III; (*k*) sucker from arm III; (*l*) sucker from median row of arm IV.

The tentacle club is the same as the larger specimen's, except that at 19.4% of the mantle length it is relatively larger and the transverse ridges of the locking zone cannot be distinguished (Fig. 1*h*). The tentacular club suckers bear 4–5 very small, blunt teeth.

The radula has five teeth in each transverse row that are not as well developed as those of the larger specimen (Fig. 1*i*).

The differences in morphology between the two specimens may be attributed to their different growth stages. Table 1 gives the measurements of the specimens.

Discussion — There are three genera of gonatid squid reported in the North Pacific: *Gonatus*, *Berryteuthis*, and *Gonatopsis*. The specimens described here appear most closely related to *Gonatus* but have one distinctive *Berryteuthis* feature. Their *Gonatus* characteristics include a radula with five longitudinal rows of teeth, a tentacular club fixing apparatus comprised of transverse ridges and suckers larger than any others on the club face, and well-developed arm hooks. They are related to *Berryteuthis* by the complete lack of hooks on the tentacular club. Larvae of *Gonatus* sp. develop the central tentacular club hook between 12 and 24 mm mantle length (Okutani and McGowan 1969; Young 1971). Since these specimens are 31 and 65 mm mantle length, it seems reasonable to accept the lack of club hooks as a permanent characteristic. In addition, the central hook is always preceded by an enlarged sucker, which is not present in these specimens.

TABLE 1. Morphometric measurements (mm) from the two gonatid squid.

	UV-TPE 65	NMC 48443
Total length (without tentacles)	123	50
Mantle length (dorsal)	65	31
Mantle width	20	12
Head length	21	10
Head width	22[a]	12
Fin length	20[a]	10
Fin width	34[a]	19
Arm length I	34	13
II	40	16
III	40	17
IV	39	16
Tentacle length — right	70	28
— left	80	36
Club length — right	10	6
— left	10	6

[a]Approximate.

A gonatid squid of this description and size has not been reported previously in cephalopod literature. However, there is a possibility that these specimens may be the same as some taken in recent collections by the *Vityaz* and other Soviet research ships (K. N. Nesis, personal communication). As yet, this similarity has not been confirmed. Okutani (1966 pl. II fig. 1, 2, pl. III fig. 1) describes a larval type from the northern North Pacific identified as Gonatidae type β, which has distinguishing characteristics similar to those given for the two specimens in question, including thick, strong arms, a muscular conico-cylindrical mantle, and tentacles that weaken in an advanced stage and bear no club hooks. Although he groups these larvae with *Gonatopsis borealis* Sasaki 1923, in which the larval form retains the tentacles but the juvenile and adult do not, Okutani states that it is still inconclusive if this type of larva with tentacles grows to a tentacle-less form. Both specimens described here have slender tentacles, but there appears to be little or no reduction in relative size and thickness from the small to the large specimen. However, the clubs are relatively smaller in the large specimen, but they are more developed in having transverse ridges in the locking zone. The Gonatidae type β larva has been collected at Weather Station "P" in the North Pacific (National Museum of Canada catalogue numbers 48449–48452). Although their general description is in keeping with that of the larger specimens, a positive relationship is difficult to establish because of the differences in size, the largest larva in the National Museum collection being 16 mm mantle length. Also, Okutani suggests that there is more than one form of the Gonatidae type β larva in the population.

Provided that larger and more mature specimens retain the characteristics described for these two specimens, it may be suggested that they represent a phylogenetic link between *Gonatus* and *Berryteuthis*. Two other generic links in the family Gonatidae have been suggested. Okutani (1967) describes a specimen of *Gonatopsis* sp. as having retained a remnant of a degenerated tentacle. He states that if this appendage is normal, *Gonatopsis* sp. may be a phylogenetic link between *Gonatus* and *Gonatopsis*. Likewise, in discussing *Gonatopsis japonicus*, Okiyama (1969) says that it may be a form of *Gonatopsis* most closely related to *Gonatus fabricii* since it has two *Gonatus* features: a radula with five longitudinal rows of teeth and a postgladial tail. These records indicate the necessity for a revision or redefinition of the genera of the family Gonatidae.

Acknowledgments — We wish to thank Dr E. Barraclough of the Fisheries Research Board of Canada, Nanaimo, B.C., for providing us with the cephalopod specimens collected on the 1969 transpacific cruise of the *Endeavour* and Dr A. H. Clarke of the National Museum of Canada, for the loan of the cephalopod specimens collected at Weather Station "P" in the North Pacific. The authors are grateful to Dr W. G. Pearcy of Oregon State University for the loan of *Gonatus* (*Berryteuthis*) *anonychus* specimens and to Dr R. E. Young of the University of Hawaii, for advice on the family Gonatidae. Work at the University of Victoria was supported by NRC Grant A 1968 and a grant from the University of Victoria Faculty Research Fund.

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Temperature Selection of Rainbow Trout (*Salmo gairdneri*) Fingerlings in Vertical and Horizontal Gradients

R. W. MCCAULEY AND W. L. POND

Department of Biology
Waterloo Lutheran University, Waterloo, Ont.

MCCAULEY, R. W., AND W. L. POND. 1971. Temperature selection of rainbow trout (*Salmo gairdneri*) fingerlings in vertical and horizontal gradients. J. Fish. Res. Bd. Canada 28: 1801–1804.

Preferred temperatures of underyearling rainbow trout (*Salmo gairdneri*) were determined in both vertical and horizontal temperature gradients. No statistically significant difference was found between the preferred temperatures by the two different methods. This suggests that the nature of the gradient plays a lesser role than generally believed in laboratory investigations of temperature preference.

MCCAULEY, R. W., AND W. L. POND. 1971. Temperature selection of rainbow trout (*Salmo gairdneri*) fingerlings in vertical and horizontal gradients. J. Fish. Res. Bd. Canada 28: 1801–1804.

La température préférée de truites arc-en-ciel (*Salmo gairdneri*) de moins d'un an a été déterminée à l'aide de gradients thermiques verticaux et horizontaux. Les températures préférées furent les mêmes avec les deux méthodes. Il semble donc que la nature du gradient n'est pas si importante qu'on l'a généralement cru dans les études en laboratoire des températures préférées.

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DETERMINATIONS of preferred or selected temperatures of fish in the laboratory are carried out by observing their movements in continuous temperature gradients established usually vertically in either deep large aquaria or horizontally in long troughs. Other kinds of apparatus are sometimes used (Fry 1958). Since there are fundamental differences in geometry between vertical and horizontal gradients, comparisons of values of preferred temperature among species have been made with reservation (Ferguson 1958; Zahn 1962). The only published comparison of preferred temperature of the same species as determined in both types of gradients is that of Javaid and Anderson (1967) who found a wide discrepancy between their own results for rainbow trout (*Salmo gairdneri*) fingerlings obtained in a horizontal gradient and those of Garside and Tait (1958) who used overyearlings in a vertical gradient tank. The most divergent values occurred at the 20 C acclimation level, the trout tested in the horizontal gradient selecting 23 C in contrast to 12 C selected by those in the vertical gradient. Javaid and Anderson suggested that the disagreement of the results of the two studies might be attributed to the different kinds of gradients. We investigated this suggestion by determining the selected temperature of a sample of trout in both vertical and horizontal temperature gradients.

Materials and Methods — Underyearlings, 12-weeks old and 4–5 cm long, were obtained in mid-July 1970, from Campbellville hatchery where they had been reared at 15–20 C for at least 1 month prior to their transfer. In the laboratory they were held at 18 C for 1 day in recirculated filtered water, after which time experiments were begun. They were fed twice daily on Silver Cup pelleted food.

Vertical gradient tank — The tank was a modified facsimile of that used by Garside and Tait who in turn had altered slightly the original design of Brett (1952). It differed mainly from previous models in that four of the five sides consisted of painted marine plywood and the fifth of transparent plexiglass through which the fish were observed. Gradients were established by adjusting the rate of downward flow of hot water through a painted spiral copper coil suspended in the tank. A source of chilled water was also available for introduction into the bottom of the tank. Temperatures of each of nine depth zones of equal width were read directly from suitably spaced thermometers that formed the rungs of a Jacob's ladder hanging in the water close to the plexiglass front.

Horizontal gradient tank — This tank, also constructed from plywood, was based on the design of Zahn (1960).[1] It consisted of a rectangular trough 15 × 15 × 150 cm through which a stream of cold water was progressively warmed by an immersion electric heater running the length of the bottom of the trough and covered by 2 cm of gravel. Horizontal thermal gradients were produced by varying the flow of water through the channel and controlling the temperature of the effluent by means of a contact thermometer, which controlled heating thermostatically through a relay. By suitable adjustment of water flow and heating, near linear gradients spanning 8 degrees C could be obtained. Vertical stratification, which sometimes arises in horizontal gradients, was largely avoided by bubbling air through air breakers placed along the side, one in each of the nine cells, of equal width. The standpipe conducting the effluent away was adjusted to provide a depth of water of 10 cm. Observations of the fish were facilitated by a mirror running the length of the trough at an angle of 45° (see Javaid and Anderson 1969).

Procedure — Seven trout were chosen at random for each experiment and transferred directly into a partially formed temperature gradient. Determinations in the horizontal gradient trough were carried out in the late afternoon and early evening of the first day and those in the vertical gradient some 24 hr later. Experiments were conducted under conditions of normal laboratory illumination consisting of fluorescent tubes on the ceiling. Details of the method of making observations have been described previously (McCauley and Tait 1970).

The method in general consisted of tabulating the positions of fish in each of the nine cells at intervals of 30 sec over a period of 10 min (designated a "run"), after which time the temperature gradient was changed and the procedure repeated.

The average temperature of each cell during a run was calculated from initial and final temperatures of each cell as read from the thermometers. Since it was virtually impossible to eliminate completely all gradients in any given space (e.g., light, gravity), the role of temperature as the main factor controlling the distribution of fish in the tanks was investigated by varying gradients and noting corresponding shifts in the distribution of fish relative to temperature zones within the gradient. Frequency polygons describing the distributions of fish in chronological sequence of gradients were plotted, from which modal-selected temperatures were determined by inspection.

The data of runs for which the widest range of temperature was available were pooled and mean temperatures along with standard deviation were calculated.

Results and discussion — The fish responded quickly and precisely to temperature in both kinds of gradients, congregating within the first half-hour in the 17–20 C range. The modal position of the distributions moved in sympathy with the location of this range in the tanks (Fig. 1). No difference was observed between mean values of preferred temperatures calculated from percentage

[1]Dr Zahn has kindly granted permission to the authors to send an English translation of his paper to anyone requesting it.

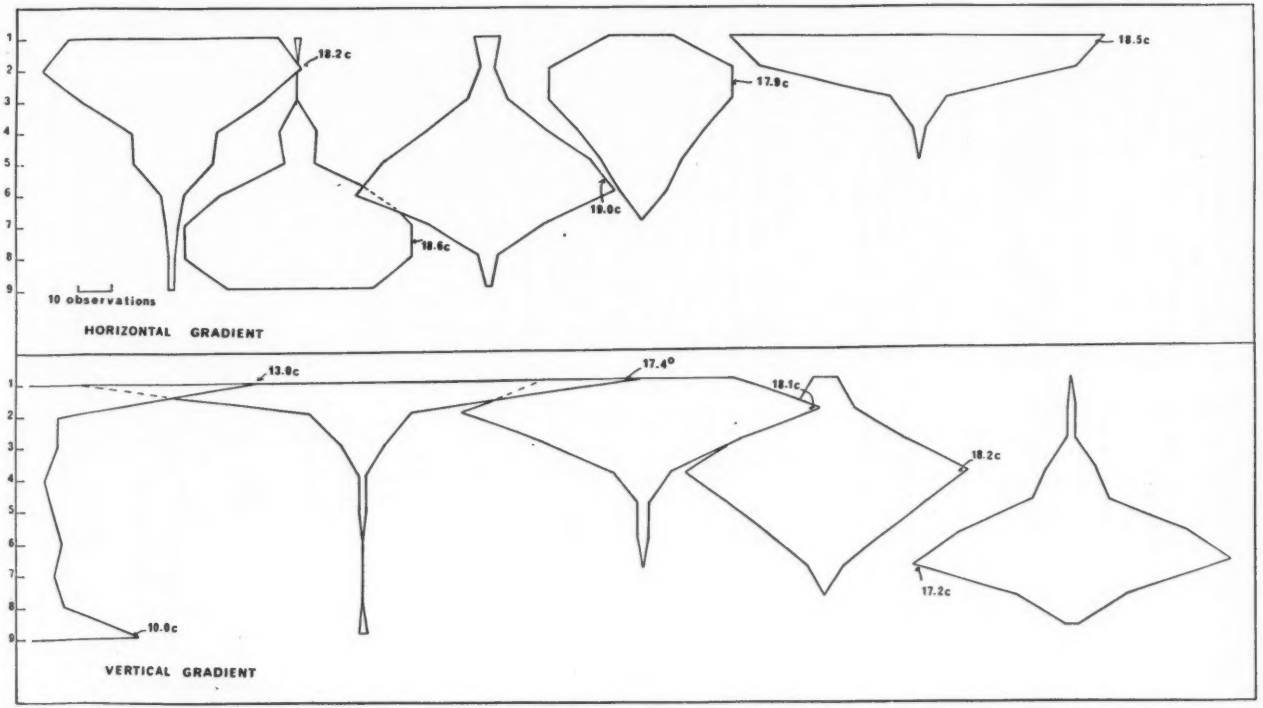

FIG. 1. Distribution of rainbow trout fingerlings in horizontal and vertical gradient apparatuses. Modal temperatures are indicated in each of the polygons.

frequencies at various temperatures (Table 1). These results indicate that, at least for rainbow trout, the nature of the gradient has little effect on the values of preferred temperature determined in the laboratory. No explanation, however, is apparent for the conflicting results of the previous two studies mentioned. At first glance it might appear that the decreased preferred temperatures for the species found by Garside and Tait (1958) is a result of the age of the fish (adults) used, since there is some evidence that adults seek colder temperatures than juveniles (Ferguson 1958). However, detailed observations of Horak and Fanner (1964) on the other hand, on the midsummer vertical distribution of adult rainbow trout in a lake, show that they favour the temperature range 17–20 C, which is more in agreement with the temperature preference of juveniles indicated by the present study.

TABLE 1. Percentage frequency distributions of rainbow trout in horizontal and vertical gradients.

Temp (*C*)	Horizontal gradient	Vertical gradient
28		.8
27		8.2
26		
25		
24		
23		
22		2.6
21	2.5	2.0
20	15.2	9.7
19	34.5	5.2
18	25.4	31.5
17	17.2	22.2
16	4.4	9.0
15	.8	6.5
14		.8
13		1.3
12		.2
Total observations	373	384
No. fish	7	7
Preferred temp (C)		
Mode	19.0	18.0
Mean	18.4	18.4
SD	1.2	2.7

Acknowledgments — This study was carried out with funds originating in part from the Freshwater Institute of the Fisheries Research Board (Winnipeg) and the Ontario Sportsmen's Show.

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Estimating Distances Between Fish Schooling in an Aquarium

PHILIP E. K. SYMONS

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

SYMONS, P. E. K. 1971. Estimating distances between fish schooling in an aquarium. J. Fish. Res. Bd. Canada 28: 1805–1806.

Distances between fish schooling in an aquarium can be estimated easily from simultaneous photographs, one taken from overhead and the other from the side. No special equipment is required, and the method is accurate to within 2 or 3 mm.

SYMONS, P. E. K. 1971. Estimating distances between fish schooling in an aquarium. J. Fish. Res. Bd. Canada 28: 1805–1806.

Il est facile d'évaluer la distance entre les membres d'un banc de poissons en aquarium en se servant de photographies simultanées, l'une prise d'en haut et l'autre de côté. La méthode, qui ne requiert aucun appareillage spécial, est précise à 2 ou 3 mm près.

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PROBABLY all studies of schooling behaviour of fish depend directly or indirectly upon an estimation of the distance or spacing between neighbours. Cullen et al. (1965) developed two methods for estimating distances through three dimensions using photographs taken from above. Their techniques require special environmental conditions such as a flat light-coloured bottom and bright light to create shadows, or special equipment such as stereoscopic camera lenses. Their methods can be used in the field, but errors up to 6 cm occur when conditions are less than optimal. This note describes a method of measuring distances through three dimensions in laboratory aquaria. No special equipment is required, and the method is accurate to within 2 or 3 mm.

Description — The method is based on the principle that the distance between two fish is the hypotenuse of a right-angled triangle whose base lies in the two horizontal dimensions, and whose side is the vertical dimension (Fig. 1). Therefore, if the length of the base of the triangle is recorded by photographing the fish school from above, and the length of the vertical is photographically recorded simultaneously from the side, the hypotenuse can be calculated by Pythagoras' theorem as the square-root of the sum of the squared lengths of base and height of the triangle between the two fish.

The only equipment required is an aquarium with one glass wall, and two cameras with a mechanism allowing simultaneous release of the two shutters; a pneumatic bulb connected by rubber hoses to the two release buttons is adequate. The cameras do not have to be equidistant from the center of the aquarium. The farther the cameras are from the aquarium, the less is the effect of refraction at water, glass, and air interfaces, but this advantage is offset by a need for greater enlargement of the photographs with resulting loss of sharpness. In this study, cameras were placed approximately 1.8 m from the center of the aquarium to cover a width of field of about 2 m (35°). Prints made from 35-mm film were enlarged to 5 × 7 inches (12.7 × 17.8 cm).

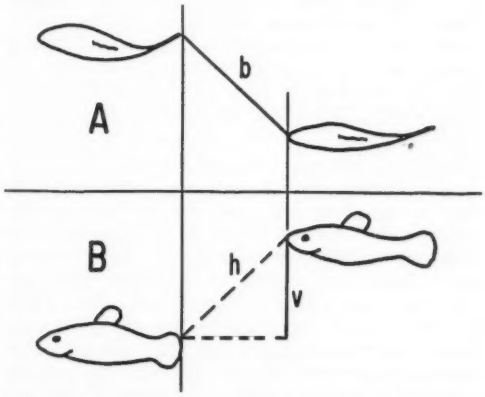

FIG. 1. Two fish as seen from above, A, and from the side, B. To calculate h from the nose of the following fish to the tail of the leader, a right-angle triangle is envisaged, as in B, where the length of the base b is measured from the overhead photograph, and the length of the vertical v from the side photo. Then $h = \sqrt{b^2+v^2}$.

Printed in Canada (J2152)

From the photographs, horizontal and vertical distances between fish images are measured with callipers. These distances must be corrected to actual distances before other calculations are made. The correction factor can be calculated from a ruler in the aquarium, from points or corners in the aquarium separated by known distances, or if the fish are of similar length, from the fish themselves. In the last instance, different correction factors for each pair of fish can be calculated if the distance between fish and cameras varies greatly between pairs. I used a correction factor calculated from the average midpoint of the schools. When measurements from the photographs have been corrected, the length of the hypotenuse is calculated to give the estimated distance through three dimensions between each pair of fish.

In studies of schooling behaviour each fish is usually paired with its nearest neighbour. To find the nearest neighbour from simultaneous photographs, distances to more than one neighbour must sometimes be calculated to find the nearest. Distances may be estimated from points between fish's eyes (Cullen et al. 1965) or from the nose of a fish to the nearest part of its nearest neighbour (Symons 1971).

Distances calculated from nose to nearest part of nearest neighbour will be shorter on the average than distances between heads or noses of fish. Distances between fish are sometimes stated in fish-lengths (Breder 1954) to facilitate comparisons between schools of large and small fish.

Determination of error — The present method was used to estimate distances between "model fish" spaced at known distances in an aquarium 200 × 48 × 35 cm deep, to determine error. The school of model fish was composed of 31 black globules (5-mm diam) of wax, each on the end of a glass rod supported in a rubber stopper. The glass rods varied in length by a maximum of 165 mm. The model fish were grouped about the aquarium in roughly the same positions as had been observed among schooling mummichog (*Fundulus heteroclitus*). The vertical distance between globules averaged about two-fifths of the horizontal distance as calculated later from photographs. Distances between globules were first measured with callipers to within 1 mm. The aquarium was then filled with water, photographed, and distances were estimated in the manner described for live fish. The process was replicated three times with different arrangements of model fish.

Within each replicate the average error, or difference between actual and estimated distances for each pair of model fish, ranged from 2.6 to 2.9 mm when calculated without regard to sign. However, when the sign was taken into account, errors tended to cancel so that average estimated and actual distances for each replicate differed by only 0.1–2.4 mm:

Avg actual distance (*mm*)	Avg distances estimated from photographs (*mm*)
71.7	73.0
72.6	72.5
60.6	58.2

With respect to mummichog (mean length about 6.7 cm), this is an average error of only 0.15–3.6%.

Some workers (van Olst and Hunter 1970) have used overhead photographs alone for calculation of distance to nearest neighbour. The reliability of this method was checked in the present study. Not always could the nearest neighbour be correctly identified from overhead photographs alone, the estimates were always less than actual distances, and the error between average estimated and actual distances of model fish ranged from 13.4 to 16.9 mm or more than 20% of a mummichog body-length. An error of similar magnitude was found when estimates from overhead photographs of live fish were compared with estimates from simultaneous photographs. Therefore, where the vertical component of spacing is as much as two-fifths or more of the horizontal component, the vertical dimension must be taken into account if a reliable estimate of distance to nearest neighbour is to be obtained. In the laboratory this can be done easily and accurately by using two cameras simultaneously.

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Mobilization of Mercuric Sulfide from Sediment into Fish under Aerobic Conditions

D. C. GILLESPIE AND D. P. SCOTT

Fisheries Research Board of Canada
Freshwater Institute, Winnipeg, Man.

GILLESPIE, D. C., AND D. P. SCOTT. 1971. Mobilization of mercuric sulfide from sediment into fish under aerobic conditions. J. Fish. Res. Bd. Canada 28: 1807–1808.

A system for studying the aerobic mobilization of mercury from aquatic sediments into fish was described. Using this method, it was shown that mercuric sulfide in sediment was very slowly mobilized and picked up by fish.

GILLESPIE, D. C., AND D. P. SCOTT. 1971. Mobilization of mercuric sulfide from sediment into fish under aerobic conditions. J. Fish. Res. Bd. Canada 28: 1807–1808.

Nous décrivons une méthode d'étude de la mobilisation aérobie du Hg dans les poissons à partir des sédiments aquatiques. Nous démontrons, à l'aide de cette méthode, que le sulfure mercurique du sédiment n'est que très lentement mobilisé et absorbé par les poissons.

Received May 21, 1971

POLLUTION of the aquatic environment with mercury and the subsequent high concentrations in fish have resulted in the need for model experiments to examine the process of transfer of mercury from sediments to fish.

Mercuric sulfide is of particular importance since low-grade cinnabar deposits are scattered throughout Canada (Jonasson 1970), and precipitation of the mercury in chlorine plant effluents as the sulfide, has been used on the basis that the very low solubility of mercuric sulfide would render it inert in the environment (Bligh MS 1970).

Jernelöv (1968) speculated that mercuric sulfide would become mobilized only after oxidation to the sulfate, a process requiring aerobic conditions. The apparatus shown in Fig. 1 was designed to study the mobilization of mercury compounds in sediments under aerobic conditions.

The system consisted of a 40-liter aquarium, a heater, and a fan to provide aeration without stirring up the sediment, which would occur with a sparging aerator. Evaporation losses were fairly high in this system, and water levels were maintained by addition of distilled water. The sediment layer in each aquarium was approximately 5 mm deep. A layer of this depth should allow oxygen penetration as long as the oxygen content of the overlying water is adequate. Periodic determinations of oxygen content of the water using an oxygen electrode showed levels of 7.5–8.6 ppm, which is close to saturation at the temperature range of 21–23 C.

Efficiency of aeration and mixing were tested visually by placing a solution of fluorescein dye on the surface of the water. The current patterns mixed the dye uniformly within 15 min and showed rapid circulation to the bottom and corners of the tank. If the tank cover was omitted, mixing and distribution were much less thorough.

For the test, a sediment of low mercury content (0.24 ppm dry weight basis) was divided into 250-g portions: mercuric chloride in 125 ml water was mixed with one sample to yield a concentration of 50 ppm mercury (dry weight basis); mercuric sulfide to 50 ppm was blended with another portion after dispersion in 125 ml water, using an 150 W ultrasonic probe; the third portion was moistened with 125 ml distilled water. After standing for 18 hr, the sediments were washed into the aquaria with tap water and the tanks equilibrated for 1 week to allow the sediments to settle. Fifty male guppies (*Poecilia reticulata*) were placed in each tank; five fish were removed from each aquarium immediately, rinsed in distilled water, and frozen for zero time mercury analysis by atomic absorption spectrophotometry (Uthe et al. 1970). Five fish were removed from each tank at each sampling time and treated similarly. Male guppies were used because they were of a convenient size for individual analysis of the whole fish. The fish were fed once a day with a minimal amount of flake tropical fish food (Tetramin), which contained 0.11 ppm on a dry weight basis.

The mercury analyses (wet weight basis) are shown graphically in Fig. 2, each point the average for five fish. As might be expected, there was a rapid increase in the levels of mercury found in fish exposed to sediments containing mercuric chloride. That this resulted from passive transfer by solution seems unlikely, since extraction of a sample of the sediment with 0.3 N HNO_3 (10 ml/g) resulted in solubilizing 1% of the contained mercury. Presumably the mercury was released slowly to the water

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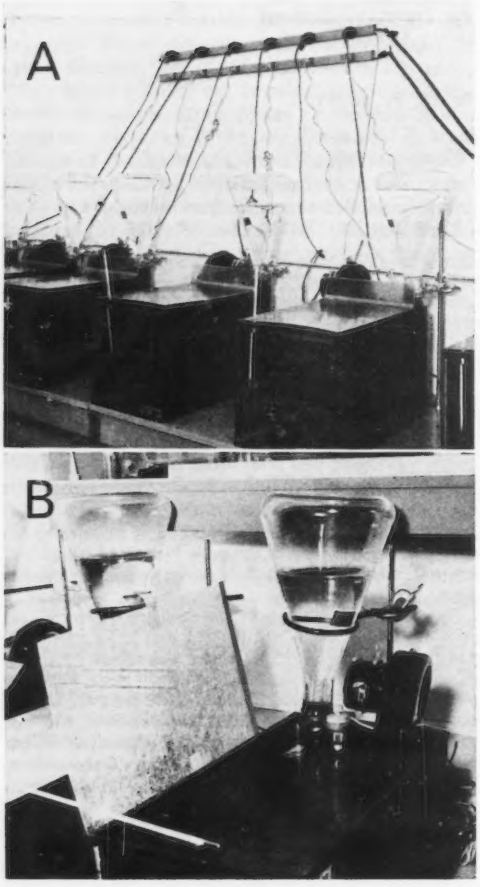

FIG. 1. Experimental system for mercury mobilization studies. A, General view, showing placing of tank lids. B, Close-up, showing arrangement of water bottle, heater, and fan.

by microbial activity in the sediments and was absorbed by the fish. The levels in fish exposed to the control sediment were surprisingly high considering the short term of the experiment. In a closed system, however, all the mercury in the food and that mobilized from the sediment would be available for absorption by fish.

Only an insignificant portion of the mercury in the sediments was found in the fish. In the control

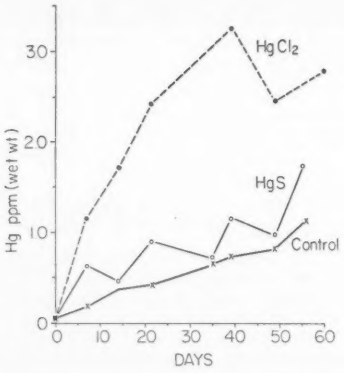

FIG. 2. Mercury levels in guppies exposed to sediments containing mercury. Each point is the average for five fish.

tank 2.3 µg of mercury was found in the fish out of 600 µg in the sediment. For the mercuric sulfide and mercuric chloride experiments 3.8 and 7.5 µg, respectively, were found in the fish out of 13,100 µg in the sediment. The added mercurials were proportionately much less rapidly mobilized than was the preexisting mercury during the short term of this experiment.

Acknowledgments — We thank Mr F. A. J. Armstrong and Dr J. F. Uthe for arranging mercury analysis on the sediments and fish.

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New Records of Lymphocystis Disease in Four Eastern Pacific Fish Species

JOHN E. MCCOSKER

Scripps Institution of Oceanography
University of California, San Diego, La Jolla, Calif. 92037, USA

AND ROSS F. NIGRELLI

Osborn Laboratories of Marine Sciences
New York Aquarium, Brooklyn, New York 11224, USA

MCCOSKER, J. E., AND R. F. NIGRELLI. 1971. New records of lymphocystis disease in four eastern Pacific fish species. J. Fish. Res. Bd. Canada 28: 1809–1810.

Four new records of lymphocystis viral disease are described for gobiid and diodontid fishes. Spontaneous infection was found in *Diodon holocanthus* and *Gobiosoma paradoxum* from the Gulf of Panamá and in *Gillichthys seta* from the upper Gulf of California. Infection was intragenerically transferred to *Diodon hystrix* in a cohabitation experiment.

MCCOSKER, J. E., AND R. F. NIGRELLI. 1971. New records of lymphocystis disease in four eastern Pacific fish species. J. Fish. Res. Bd. Canada 28: 1809–1810.

Nous décrivons quatre nouveaux cas de maladie à virus lymphocystique découverte chez des poissons de la famille des Gobiidae et des Diodontidae. Nous avons observé une infection spontanée chez *Diodon holocanthus* et *Gobiosoma paradoxum* du golfe de Panama et *Gillichthys seta* du secteur nord du golfe de Californie. Au cours d'une expérience de cohabitation, la maladie a été transmise à une autre espèce, *Diodon hystrix*.

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SPONTANEOUS lymphocystis viral disease has been recorded from 51 species in 21 families and 5 orders of fishes (Nigrelli and Ruggieri 1965; McCosker 1969). The discovery of three additional hosts to this spontaneous infection and the experimental induction in an additional host species have prompted this note.

Recent collections of shallow-water fishes in the Gulf of California and in Pacific Panama have disclosed the first observations of lymphocystis infection in the family Gobiidae. A 64.0-mm standard length (SL) specimen of *Gillichthys seta* (Ginsburg) collected in the upper Gulf of California constitutes the second infected species from that body of water. Characteristic lymphocystis tumors were located on the left pectoral and dorsal fins, with a few small cysts on the flanks. The specimen (Osborn Laboratories No. 69–1962, formerly SIO 69–198) was collected with quinaldine in shallow subtidal water (0–2 m) offshore from the Puertecitos refuse dump, 2 km north of Puertecitos, Baja California Norte, Mexico, by J. E. McCosker and S. Smith on March 23, 1969. A second infected gobiid, *Gobiosoma paradoxum* (Gilbert), had lymphocystis tumors (120–300 μ in diam) on all fins and fin membranes and a few isolated cysts on the flanks. The 25.5-mm SL specimen (JEM 71–12) was collected in the feeder stream to a shallow, low-salinity estuary at the Diablo Heights Boat Dock, Pacific entrance to the Panamá Canal, by J. E. McCosker on February 9, 1971.

Eight spiny puffers, *Diodon holocanthus* Linnaeus (78–125 mm SL), trapped in Panamá Bay during November and December 1970, displayed cystlike lesions of the body characteristic of lymphocystis, the ubiquitous viral disease of freshwater and marine fishes. This species, previously unrecorded as a susceptible host, was collected during a regular trapping program carried out since August 1970 from the Smithsonian Tropical Research Institute pier (trap depth 3–10 m) on Naos Island, Panamá Bay, Panamá (8°55′10″N, 79°32′10″W). The spiny puffer, generally infrequently caught, became prevalent in mid-November and continued until late December. The appearance of the lymphocystis-infected individuals (eight of 21 fish examined, or 38%) coincides with the end of the Gulf of Panamá wet season.

The lymphocystis lesions in *Diodon* were located on the pectoral fins, in the skin of the spines, and in the skin of the dorsal part of the body. The diagnosis was verified microscopically by the

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presence of numerous encapsulated hypertrophied cells, each containing the characteristic basophilic substance in the cytoplasm (Fig. 1, 2). All stages of enlargement, 15–500 μ, were present in the loose connective tissue of the subdermal region. The tissue around the disease cells was infiltrated with macrophages and an unusual number of involuted plasma cells with pycnotic eccentric nuclei and acidophilic substances in the cytoplasm suggestive of Russell's Bodies (Fig. 3).

The lymphocystis disease, although highly infectious, is nevertheless nonlethal, and (except for the presence of another but mild infection with *Trichodina*, a common ciliate parasite of the gills) the spiny puffers showed no behavior indicative of morbidity. The typical *Diodon* defense posture of inflating and erecting the body spines perhaps facilitates the passage of the virus; when the body is fully inflated, the spines often break through the epidermis, forming foci for the invasion of the lymphocystis virus. Other benthic species occupying a similar habitat and trapped concomitantly (*Arothron hispidus*, *Canthigaster punctatissimus*, *Lutjanus* spp., *Alphetes multiguttatus*, and *Epinephelus* spp.) were not infected.

Lymphocystis virus was experimentally passed from *Diodon holocanthus* to the porcupine fish, *D. hystrix* Linnaeus. A 160-mm SL *D. hystrix* was tagged and placed in a running seawater aquarium (temperature range 25.5–28.0 C, salinity 26.5–29.5‰) along with four infected *D. holocanthus* (95–125 mm SL). Lymphocystis lesions appeared on the dorsal fin of *D. hystrix* after 15 days and were well developed on the dorsal and left pectoral fins in 30 days.

The possibility that this individual was harboring a prior systemic infection was ruled out in that six *D. hystrix* (ca. 150–300 mm) collected from the same locality during October and November 1970 showed no indication of the virus. A similar cohabitation transfer experiment in an adjoining tank using other tetraodontiform fishes (*A. hispidus*, *Sphoeroides annulatus*, *C. punctatissimus*, and *Balistes naufragium*) as possible hosts for the lymphocystis virus was unsuccessful. These qualitative experiments agree with Weissenberg's (1939) and Wolf's (1962) findings that lymphocystis transfer is most effective when closely related species are used as host and donor.

Acknowledgments — The senior author's work was done during his tenure as a predoctoral intern with the Smithsonian Tropical Research Institute whose support is graciously acknowledged.

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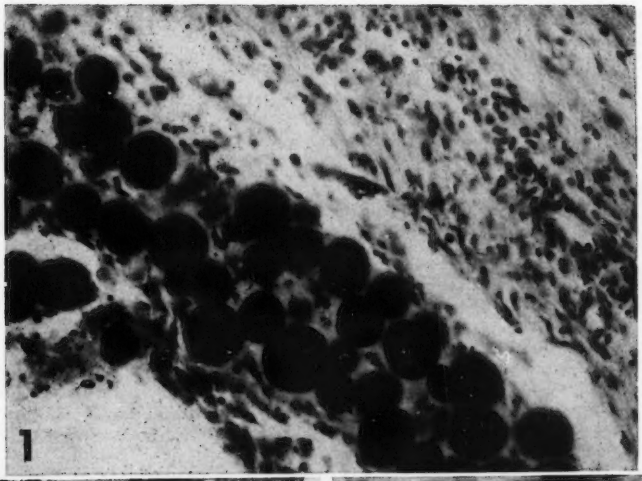

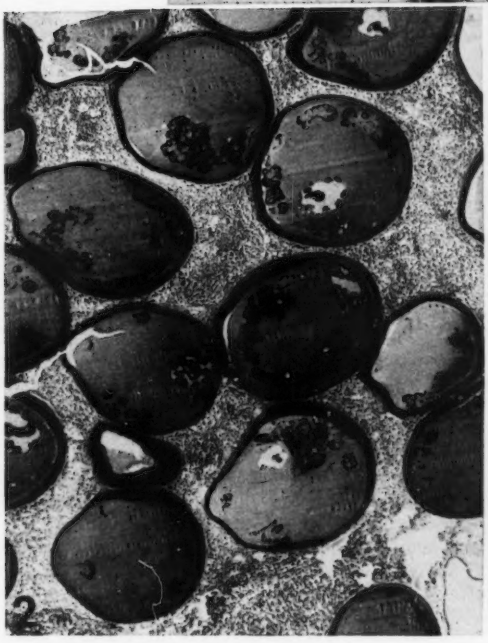

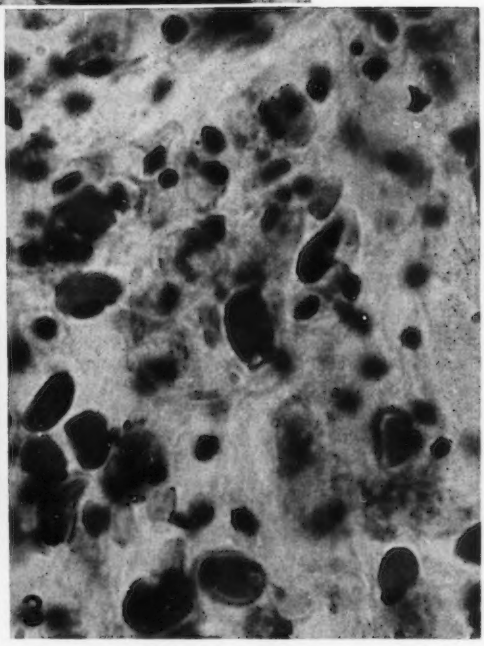

FIG. 1. Cluster of "young" lymphocystis cells in the subdermal tissue of a lesion in the spine of *Diodon*. Giemsa. 450×.

FIG. 2. Group of enlarged (mature) cells showing characteristics typical of viral lymphocystis disease. Giemsa. 120×.

FIG. 3. Clusters of involuted plasma cells with acidophilic substance in the cytoplasm reminiscent of Russell's Bodies in higher vertebrates. Giemsa. 1000×.

Survival of Some Freshwater Fishes in the Alkaline Eutrophic Waters of Nebraska

D. B. McCarraher

Nebraska Game and Parks Commission
Office of Limnology, Hastings College, Hastings, Nebraska 68901 USA

McCarraher, D. B. 1971. Survival of some freshwater fishes in the alkaline eutrophic waters of Nebraska. J. Fish. Res. Bd. Canada 28: 1811–1814.

Of 14 species of freshwater fishes held in cages in one or more of 13 alkaline lakes and ponds in Nebraska, few species survived more than a month where carbonate alkalinity, mostly as compounds of Na_2CO_3 and KCO_3, was above 300 mg/liter. Of the 14 species tested, Sacramento perch (*Archoplites interruptus*), fathead minnow (*Pimephales promelas*), northern pike (*Esox lucius*), and the black bullhead (*Ictalurus melas*), were the most tolerant of alkaline environments. Most centrachid fishes except *A. interruptus* and the green sunfish (*Lepomis cyanellus*), did not survive more than a month in alkaline waters greater than 950 mg/liter total alkalinity. A lake classification index for slightly alkaline to strongly alkaline environments is presented, along with suggested fish species for introduction into such waters.

McCarraher, D. B. 1971. Survival of some freshwater fishes in the alkaline eutrophic waters of Nebraska. J. Fish. Res. Bd. Canada 28: 1811–1814.

Sur un total de 14 espèces de poissons dulcaquicoles maintenus en cages dans un ou plusieurs de 13 lacs et étangs alcalins du Nebraska, peu d'espèces survécurent plus d'un mois là où les carbonates alcalins, surtout des composés de Na_2CO_3 et KCO_3, dépassaient 300 mg/liter. Des 14 espèces soumises aux essais, la perche de Sacramento (*Archoplites interruptus*), le méné à grosse tête (*Pimephales promelas*), le grand brochet (*Esox lucius*), et la barbue noire (*Ictalurus melas*), se sont avérés les plus tolérants pour les milieux alcalins. La plupart des centrarchidés, sauf *A. interruptus* et le crapet vert (*Lepomis cyanellus*), n'ont pu vivre plus d'un mois dans des eaux d'alcalinité totale excédant 950 mg/liter. On présente un index de classification des lacs, de légèrement à fortement alcalins, et on suggère les espèces à introduire dans chacun de ces milieux.

Received December 14, 1970

Past investigations into the survival of freshwater fishes in the inland mineral waters of North America have been directed mainly toward the chloride–sulfate type of habitat (Rawson and Moore 1944). This report on inland mineral lakes describes water quality characteristics of 13 lakes of various alkalinities scattered throughout the 52,000 km^2 sandhill region of Nebraska. Field investigations were conducted from 1960 to 1967 to determine the survival of 14 fish species in one or more of the 13 lakes. Based partially on these experimental results, a list of fish species is suggested for introduction into slightly alkaline to strongly alkaline environments.

The ecological relation between alkalinity and northern pike (*Esox lucius*), fathead minnow (*Pimephales promelas*), and the Sacramento perch (*Archoplites interruptus*), along with a general limnological assessment of these lakes was described earlier (McCarraher unpublished data 1962; McCarraher and Thomas 1968; McCarraher and Gregory 1970).

Methods and materials — Some of the experimental fish (*Micropterus salmoides, M. dolomieui, Ambloplites rupestris, Ictalurus punctatus, Morone americana, M. chrysops*, and *Pomoxis nigromaculatus*) were taken from hatchery stock and selected for robustness and freedom from external parasites. The remaining species (*E. lucius, Stizostedion vitreum vitreum, Cyprinus carpio, Lepomis cyanellus, Perca flavescens, Ictalurus melas, A. interruptus*, and *Aplodinotus grunniens*) were captured by seines and frame nets from freshwater lakes. All species were either age I or II and were chemically–thermally tempered to lake water for 2–4 hr.

The fish, except those of two species, were then released into 1.1-m^3 cages constructed of 8-mm mesh galvanized wire, and the cages anchored near shore in about 0.5–1 m depth. The cages were examined each hour for the first 6 hr and dead or distressed fish removed. If the fish survived the initial 48-hr period, the cages were then checked every 2–3 days. That mortality of the experimental fish was due to starvation rather than the direct influence of alkaline

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TABLE 1. Survival of some freshwater fishes and related water chemistry, in milligrams per liter, in Nebraska lakes of various alkalinities.

Lake	Survival time[a]	Total alkalinity range	Average CO_3 alkalinity	Total dissolved solids	*p*H	Cl	SO_4	K	Na	Ca	Total hardness
				Northern pike							
Big Alkali	Indefinite	460–950	130	1120	9.3	20	14	90	210	38.0	50
Smithys Pond #1	36–48 h	886–880	254	9230	10.5	235	600	–	4200	50.0	12
Hudson	12–14 m	820–1250	290	1170	9.5	30	30	300	350	20.0	200
Smithys L.	50–58 h	2035–2050	769	3449	9.8	140	120	1800	1600	35.0	120
Smithys Pond #2	6– 8 h	3936–3940	960	11600	10.8	300	860	–	3200	0.0	140
Little Alkali	6–12 h	4500–4508	1139	6250	10.3	350	344	–	1184	4.0	10
				Walleye							
Big Alkali	2– 4 y	300– 980	85	860	9.3	17	12	80	120	30.0	42
Smithys L.	10–18 h	1890–1895	714	2315	9.6	160	40	750	1000	48.8	114
McKeel Pond #2	28–30 d	1921–2060	680	2980	9.4	140	40	1200	1100	59.0	152
Little Alkali	4–10 h	2810–2810	940	2130	9.7	–	–	660	720	–	20
				Black bullhead							
Walgren	Indefinite	380– 700	110	380	8.5	8	4	4	18	102.0	329
Smithys L.	30–33 d	1802–2160	620	2050	9.5	80	38	700	800	50.0	130
Smithys Pond #1	18–22 h	3280–3300	1802	4320	9.9	50	160	900	1800	4.0	20
				Channel catfish							
Smithys L.	10–18 h	1890–1895	714	2315	9.6	160	40	750	1000	48.0	114
				White perch							
Walgren[b]	Indefinite	380– 700	110	380	8.5	8	4	4	18	102.0	329
Rogers	6– 8 d	1700–1780	578	1980	9.3	34	14	215	300	20.0	768
Smithys L.	18–20 h	2176–2180	918	2860	9.8	90	50	1200	1600	30.0	115
Smithys Pond #1	12–14 h	3280–3215	1800	4300	9.9	50	160	910	1800	10.0	20
				White bass							
Little Alkali	4–10 h	2810–2810	940	2130	9.7	–	–	660	720	–	20
Smithys Pond #1	6– 9 h	3706–3715	2256	5100	9.8	68	190	1900	2200	4.0	14
				Drum							
Smithys L.	10–18 h	1890–1895	714	2315	9.6	160	40	750	1000	48.0	114
				Largemouth bass & bluegill							
Big Alkali	Indefinite	300– 950	85	860	9.3	17	12	80	120	30.0	42
Stout	3– 4 m	270– 420	145	500	9.5	99	38	92	80	56.0	119
McKeel Pond #1	10–11 d	1143–1250	442	1275	9.3	100	60	250	450	40.0	2118
				Rock bass							
McKeel Pond #5	12 d	1360–1410	272	1410	9.5	80	44	200	350	52.0	80
McKeel Pond #2	28–30 d	1921–2060	680	2980	9.4	140	40	1200	1100	59.0	152
				Sacramento perch							
Walgren	Indefinite	380– 700	110	380	8.5	8	4	4	18	102.0	329
White	12–18 m	1100–2100	466	1880	9.1	29	620	187	435	33.0	48
Smithys L.	96–98 d	1906–3060	816	2223	9.5	170	38	800	1200	50.0	120
Little Alkali	22–24 d	2040–2870	1026	2850	9.7	100	120	775	740	17.0	35
				Smallmouth bass							
Little Alkali	4–10 h	2810–2810	940	2130	9.7	–	–	660	720	–	20
				Green sunfish							
East Twin	6–12 m	1430–2960	530	2830	9.5	100	43	500	655	22.0	100
				Fathead minnow							
White	12–18 m	1100–2100	466	1880	9.1	29	620	187	435	33.0	48

[a]h, hours; d, days; m, months; y, years.
[b]Western distribution limit in North America.

compounds was unlikely, as food organisms were found in the stomachs of dead fish. *Pimephales promelas* and *S. vitreum vitreum* were not confined to cages but were released directly into Big Alkali and Smithys lakes. These releases were examined periodically with gill- and frame nets.

Dissolved oxygen concentrations remained at 5.8 mg/liter or above throughout the tests. Water quality was determined by standard methods (American Public Health Association 1965). Alkalinity and *p*H values were determined immediately following the collection of water samples from lake and pond sites shown in Table 1.

Results and discussion — Of the 14 species tested, those with the most noteworthy tolerance to alkalinity were the Sacramento perch and the fathead minnow; they survived 12–18 months in lakes where the total alkalinity ranged from 1000 to 2000 mg/liter. The green sunfish, the next most tolerant species to medium alkaline waters, occurs naturally in several sandhill lakes where the maximum seasonal alkalinity does not exceed 2000 mg/liter (e.g., East Twin Lake, Table 1). The species has survived and reproduced in Skull Lake, where the alkalinity fluctuates between 400 and 960 mg/liter (McCarraher and Thomas 1968).

Northern pike survived for about 14 months in the medium alkaline waters of Hudson Lake until the total alkalinity exceeded 1200 mg/liter and the carbonate alkalinity exceeded 600 mg/liter (Table 1). In Big Alkali Lake, a pike population has survived since the early 1950's, the success of annual recruitment depending upon the alkalinity concentration of the water during the spawning season; alkalinity values in excess of 950 mg/liter normally cause extensive mortality of eggs and fry (unpublished data).

The black bullhead, *Ictalurus melas*, and the channel catfish, *I. punctatus*, revealed noteworthy differences in ability to survive. *Ictalurus melas*

TABLE 2. Some suggested fish species for introduction into alkaline eutrophic lakes of the world.

Lake type[a]	Total alk. range	Carbonate alk. range	K + Na range	Species
Slightly alkaline	<900	<250	<200	*Ambloplites rupestris*
				Aplodinotus grunniens
				Ictalurus punctatus
				Lepomis macrochirus
				Micropterus dolomieui
				Micropterus salmoides
				Morone americana
				Pomoxis nigromaculatus
Medium alkaline	900–1200	300–600	200–350	*Cyprinus carpio*
				Esox lucius
				Ictalurus melas
				Lepomis cyanellus
				Morone chrysops
				Perca flavescens
				Stizostedion vitreum vitreum
Moderately alkaline	1200–1800	550–850	350–400	*Archoplites interruptus*
				Clupeonella abrau[b]
				Fundulus kansae
				Pimephales promelas
				Tilapia aurea[b]
Strongly alkaline	1800–3000+	900–1200+	400–2000+	*Craterocephales fluviatilis*[b]
				Culaea inconstans
				Cyprinodon variegatus
				Fundulus majalis
				Mugil cephalus[b]
				Tilapia galilaea
				Tilapia aurea[b]

[a]Refers to the author's classification of alkaline waters based on his examination of about 1200 lakes in North America and Africa.

[b]Species not presently found in North American alkaline waters but may have potential for successful adaptation.

survived for a considerably longer period than *I. punctatus* in the strongly alkaline water of Smithys Lake.

The walleye, *S. vitreum vitreum*, survived up to 4 years in Big Alkali Lake, where carbonate alkalinity seldom exceeded a range between 60 and 90 mg/liter. Fingerling and adult fish can survive in Big Alkali Lake; however, natural reproduction was greatly curtailed when carbonate alkalinity values exceeded 90 mg/liter during spawning season (unpublished data). Early aquaria studies by Young (1923) using various concentrations of alkaline compounds indicated that walleye could not survive with carbonate alkalinity concentrations greater than 72 mg/liter, whereas Rawson and Moore (1944) reported that the species survived in saline lakes of Saskatchewan where the total alkalinity was about 483 mg/liter. The salts of these saline lakes are for the most part sulfates of sodium and magnesium and therefore chemically different from those of Nebraska lakes.

Channel catfish (*I. punctatus*), bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieui*), and rock bass (*A. rupestris*), were found to be unsuitable for medium to strongly alkaline waters and are suitable only for introduction into waters classified as slightly alkaline (Table 2). Aquaria experiments with alkaline water by Young (1923), Calabrese (1969), and Stiemke and Eckenfelder (1947) all indicated that the death point for *L. macrochirus* in a Na_2CO_3 solution was 120–160 mg/liter. Observations by the author (unpublished data for 1954–1965) showed that survival of *L. macrochirus* and *M. salmoides* in Big Alkali Lake depended on the carbonate alkalinity remaining below 250 mg/liter for periods greater than 3 months of the year. By contrast, Renfro (1959) has described significant survival of *M. salmoides* in noncarbonate chlorosulfate waters below 9‰ total dissolved solids with progressive lethal effects between 9 and 15‰.

Sacramento perch not only appeared to have superior survival qualities for alkaline waters but is presently found living in several of the noncarbonate saline lakes of North Dakota, Colorado, and Nevada (McCarraher and Gregory 1970).

The fathead minnow inhabits numerous alkaline–saline lakes throughout the northern Great Plains (Nebraska, South Dakota, North Dakota, and the prairies of southern Saskatchewan and Manitoba) (McCarraher and Thomas 1968). The green sunfish, together with the Sacramento perch and the fathead minnow, are the most adaptable of Nebraska's fish fauna for survival in alkaline waters.

A list of species for intorduction into alkaline waters (Table 2) is suggested on the basis of experiments in Nebraska lakes; other species, not tested, are included for consideration.

Acknowledgments — The advice and editorial assistance of Mr R. E. Thomas and the encouragement of Mr G. Foster during the course of this study is gratefully acknowledged.

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A Dispenser for Winkler's Dissolved Oxygen Reagents[1]

I. W. DUEDALL

Fisheries Research Board of Canada
Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

A. R. COOTE, D. F. KNOX, AND G. F. CONNOLLY

Department of the Environment
Marine Sciences Branch
Atlantic Oceanographic Laboratory, Bedford Institute, Dartmouth, N.S.

DUEDALL, I. W., A. R. COOTE, D. F. KNOX, AND G. F. CONNOLLY. 1971. A dispenser for Winkler's dissolved oxygen reagents. J. Fish. Res. Bd. Canada 28: 1815–1816.

A new dispenser for delivering Winkler's oxygen reagents simultaneously is described.

DUEDALL, I. W., A. R. COOTE, D. F. KNOX, AND G. F. CONNOLLY. 1971. A dispenser for Winkler's dissolved oxygen reagents. J. Fish. Res. Bd. Canada 28: 1815–1816.

Les auteurs décrivent un nouveau dispensateur à distribution simultanée des réactifs du dosage de l'oxygène suivant la méthode de Winkler.

Received July 12, 1971

DURING the *Hudson'70* Expedition, a specially designed dispenser (Fig. 1) for the oxygen pickling reagents was used in Carpenter's (1965) modification of the Winkler dissolved oxygen method. Participants of the Expedition commented on the usefulness of this dispenser; this note outlines the design features. The main feature, which was originally suggested to us by K. Grasshoff, is that the required amounts of the two pickling reagents ($MnCl_2$ and NaI–NaOH) are added simultaneously from two syringes when the dispenser crank is turned one revolution. In our procedure, a water sample is first drawn into an iodine flask and the sample is immediately brought into contact with the syringe needles of the dispenser. The flask is then raised so the needle tips are situated near the bottom of the flask. The oxygen reagents are dispensed, the iodine flask is stoppered and shaken, and the sample is analysed according to Carpenter's technique.

The dispenser (Fig. 2) consists of two 30-ml B-D®[2] plastic syringes in which the syringe barrels are fastened to an anodized aluminum stationary plate and the syringe plungers are fastened to a sliding plate; the two plates are connected by a threaded pipe having 9 threads per inch. The syringe size was chosen so that all 25 oxygen samples from one oceanographic bottle cast could be pickled

[1]B.I. Contribution 275.
[2]Becton, Dickson and Co., Rutherford, N.J.
[3]Bard-Parker Co., Rutherford, N.J.

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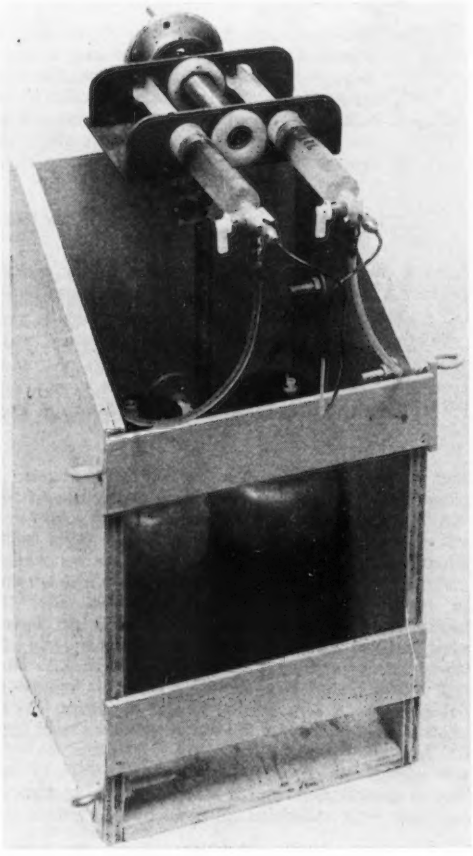

FIG. 1. The reagent dispenser.

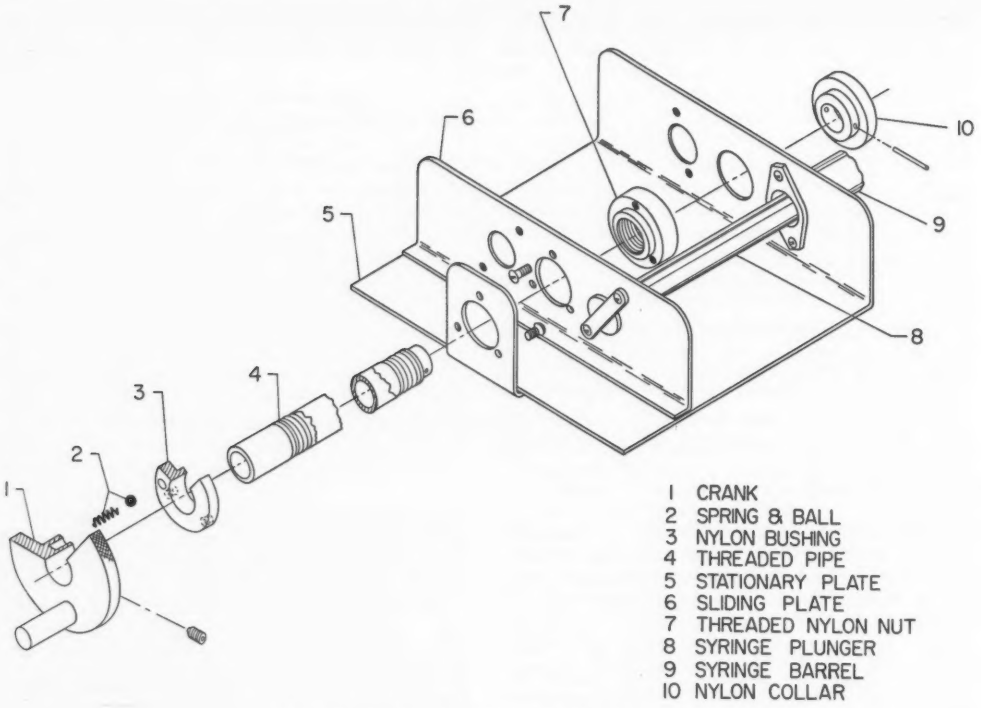

FIG. 2. An "exploded" view of the dispenser.

from a single filling. One revolution of the crank delivers 0.97 ml of each reagent with a repeatability of ±.003 ml. A spring and ball was fixed in the crank and a small hemispherical depression was ground in the nylon bushing so the operator could accurately sense one revolution of the crank. B-P®3 three-way valves (No. 3406) were fitted on the tops of the syringes to allow convenient refilling from reagent reservoirs.

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Acid Phosphatase Activity in Vegetative Cells and Spores of *Clostridium botulinum* Type E

G. A. STRASDINE AND JOANNE M. MELVILLE

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

STRASDINE, G. A., AND J. M. MELVILLE. 1971. Acid phosphatase activity in vegetative cells and spores of *Clostridium botulinum* type E. J. Fish. Res. Bd. Canada 28: 1817–1820.

Acid phosphatase activity with a *p*H optimum of 5 was demonstrated in vegetative cells, spores, and germinated spores of *Clostridium botulinum* type E (Minnesota). The enzyme was present in the cells during all stages of growth and was insensitive to the orthophosphate concentration of the growth media. Specific activity of the enzyme increased during growth coincident with a loss in inorganic phosphate from the acid-soluble cell fraction. Magnesium or manganese was required for maximum enzyme activity. Acid phosphatase in crude spore extracts was more heat-stable than in extracts obtained from vegetative cells.

STRASDINE, G. A., AND J. M. MELVILLE. 1971. Acid phosphatase activity in vegetative cells and spores of *Clostridium botulinum* type E. J. Fish. Res. Bd. Canada 28: 1817–1820.

Nous décrivons l'activité de la phosphatase acide en *p*H optimal de 5 dans les cellules végétatives, les spores ordinaires et les spores en voie de germination de *Clostridium botulinum* type E (Minnesota). L'enzyme est présente dans la cellule à tous les stades de croissance et n'est pas affectée par la teneur en orthophosphate du milieu. L'activité spécifique de l'enzyme augmente durant la croissance, coïncidemment à une perte de P inorganique de la fraction cellulaire soluble dans l'acide. La présence de Mg et Mn est nécessaire pour une activité enzymatique maximale. La phosphatase acide d'extraits bruts de spores est plus stable à la chaleur que celle d'extraits de cellules végétatives.

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ENZYMES that catalyze the hydrolysis of monophosphate esters are widespread in microorganisms. The majority of microbial alkaline phosphatases that have been studied appear to be repressible systems and respond to the available supply of inorganic phosphate (Dorn 1968). Less information is available on the metabolic function of microbial acid phosphatases. For the most part, the acid phosphatases are insensitive to phosphate repression and are present in the cell under all conditions of growth (Hofsten 1961). In view of the transferase activity attributed to some phosphatases, a more anabolic function has been suggested for these enzymes (Koshland 1959). Utilization of hexose phosphates for growth at acid *p*H has also been suggested as a possible role for these enzymes (Hofsten 1961).

In a previous study (Strasdine 1968), it was demonstrated that vegetative cells of *Clostridium botulinum* type E accumulated a glucanlike polysaccharide. Experiments designed to elucidate the mechanism of glucan biosynthesis in cell extracts of this organism revealed the presence of an active phosphatase enzyme system catalyzing the rapid liberation of orthophosphate from glucose-1-phosphate and the chromogenic phenolic ester, *p*-nitrophenyl phosphate. Some general properties of the acid phosphatase enzyme in this organism are presented in this note.

Materials and methods — *Clostridium botulinum* type E (Minnesota) was grown in trypticase–peptone media containing 0.4% glucose as described previously (Strasdine 1968). Growth at *p*H 7.0 was achieved by the controlled addition of 0.1 N NaOH to the culture by means of a syringe and "*p*H-stat" assembly (Metrohm, Herisau Switzerland). Acid phosphatase activity in whole cell and cell extract preparations was determined by measuring the absorbance at 410 nm resulting from the enzymatic hydrolysis of *p*-nitrophenyl phosphate (NPP) as described by Barnes and Morris (1957). The assay system, in a final volume of 1.0 ml, contained 0.2 ml of a suitably diluted cell suspension or cell extract; 0.6 ml buffer (Tris–maleate 0.2 M, *p*H 5.0); and 0.2 ml of NPP (50 μmoles/ml). Phosphatase activity was shown to be proportional to the concentration of cells or extract added and to the incubation time. One unit

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of enzyme activity is equivalent to the formation of 1 mμmole of *p*-nitrophenol (NP) per min at 30 C.

Vegetative cell and spore extracts were prepared by the sonic disruption (Model W185 E- Sonifier, Heat Systems Inc., New York, N.Y.) of washed cell or spore suspensions (40 × growth) for 6 min at 4 C, and collection of the supernatant fraction following centrifugation at 10,000 × *g* for 30 min at 4 C. For the disruption of spores, two parts (w/v) of teflon-coated glass beads (0.10-mm diam) were added to the spore suspension. Germinated-spore suspensions were prepared as previously described (Strasdine 1966) and contained chloramphenicol (1 mg/ml) to prevent spore outgrowth.

Total and inorganic phosphate were determined by the method of King (1932). Protein was estimated by the method of Lowry et al. (1951), and total carbohydrate by means of the anthrone reagent (Morris 1948). Acid-soluble cell fractions were prepared by extracting whole cells with 5% trichloroacetic acid as described by Roberts et al. (1957).

Observations and discussion — The acid phosphatase activity of cell-free extracts displayed a *p*H optimum in Tris–maleate buffer at *p*H 5.0 (Fig. 1). Acetic acid–sodium acetate and succinic acid–sodium hydroxide buffers gave similar *p*H optima. All subsequent assays were performed at *p*H 5.0 in Tris–maleate buffer.

Figure 2 shows the course of growth, acid-phosphatase production, and *p*H change of the media during anaerobic growth of the culture in trypticase–peptone media. The specific activity of the enzyme (units/mg protein) remained relatively constant during the early stages of growth, then increased slightly between 14 and 16 hr incubation. Maximum enzyme activity under these conditions was 0.5–0.6 units/ml of culture. If the *p*H of the culture was maintained at 7.0 by means of alkali addition, there occurred almost a twofold increase in enzyme production. Under conditions of constant *p*H, the specific activity of the enzyme increased markedly between 14 and 16 hr (Fig. 3).

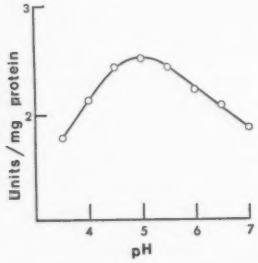

FIG. 1. *p*H optimum of the acid phosphatase activity of *C. botulinum* type E; 0.2 ml of a crude cell-free extract (9.6 mg protein/ml) was incubated with NPP in Tris–maleate buffer at 30 C.

In view of the sudden increase in the specific activity of the phosphatase during the latter stages of logarithmic growth, the inorganic (P_i) and organic (P_o) phosphorous concentrations of the media were determined at hourly intervals during growth of the culture at constant *p*H (Fig. 4). At no time did the concentration of P_i or P_o become depleted but remained relatively constant with a possible slight loss in P_o and a slight increase in P_i. Increasing the P_i concentration of the media to 25 μmoles/ml had no effect on phosphatase activity. There was, however, a rapid loss of P_i from the acid-soluble cell fraction during growth. Intracellular P_i appeared to be limiting at about 14–16 hr incubation, corresponding to the increase in specific activity of acid phosphatase. On this basis, the enzyme appears to function in the manner of a repressible system responding to the concentration of intracellular orthophosphate.

Cleavage of hexose phosphate substrates at acid *p*H has been suggested as a possible role for microbial

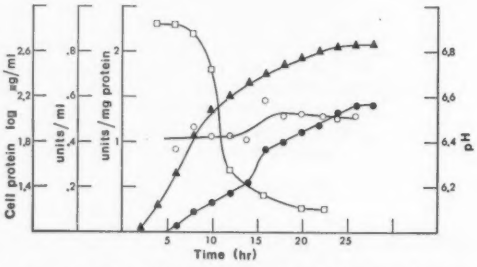

FIG. 2. Total protein, acid phosphatase production, and *p*H change during growth of *C. botulinum* type E; 0.2 ml of suitably diluted whole cell suspensions were incubated with NPP at 30 C for 30 min. Protein/ml of culture (▲), enzyme units/ml (●), enzyme units/mg protein (○), *p*H (□).

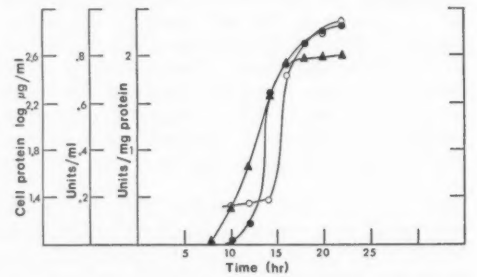

FIG. 3. Total protein and acid phosphatase production during growth at *p*H 7.0 of *C. botulinum* type E. Assay systems and legends are described in Fig. 2.

acid phosphatases (Hofsten 1961). In this respect, the Minnesota strain has been shown to accumulate and subsequently utilize a glucanlike polysaccharide during growth in trypticase–peptone media (G. A. Strasdine unpublished data). It may be seen from Fig. 3 and 4 that the increase in specific activity of the enzyme precedes carbohydrate accumulation. This may suggest a possible role for photophatase in the utilization of hexose phosphate resulting from a phosphorolysis of intracellular glucan.

Phosphatase activity could not be detected in cleaned refractile spore suspensions, but was readily detected in extracts of disrupted spores. Unlike vegetative cell extracts, in which the enzyme was associated with the soluble cell fraction (10,000 $\times$ *g* supernatant), spore phosphatase was almost completely contained in insoluble (particulate) spore material (10,000 $\times$ *g* pellet). In a typical experiment where the unfractionated spore extract contained 0.88 units of enzyme/ml, 0.68 units or 77% of the activity was contained in the spore-insoluble fraction; the remainder (0.15 units) could be accounted for in the soluble cell fraction. Increasing the sonic-disruption time increased solubilization and decreased enzyme activity. Germinated spore suspensions, in which spore outgrowth and protein synthesis were inhibited by the presence of chloramphenicol, possessed acid phosphatase activity. The total activity of germinated spore suspensions was equivalent to the total activity of spore extracts obtained from the same volume of refractile spores.

Dialysis of crude cell and spore extracts for 24 hr against distilled water reduced the phosphatase activity of these extracts by about 70–80%. Enzyme activity was completely restored by the addition of magnesium or manganese to a final concentration of 0.01 M.

A difference in thermal stability between vegetative and spore phosphatase was noted. The half-life of the spore enzyme at 65 C was approximately 3 min. The phosphatase activity in vegetative cell extracts, on the other hand, decreased 97% when held at 60 C for 1 min. The heat stability of the spore enzyme is not as high as has been reported by Green and Sadoff (1965) for the $NADH_2$ oxidase of *C. botulinum* type A (35 min at 85 C). This may be a reflection of the lower thermal stability of type E spores compared to spores of types A and B (Schmidt 1964).

Considerably more data will be required to establish an in vivo role for this enzyme(s) activity in *C. botulinum* type E. Nevertheless, some degree of speculation may be warranted at this time if only for the purpose of introducing a previous observation that may be relevant. Sporulation in the Minnesota strain is initiated during the latter stages of glucan accumulation and coincides with the exhaustion of exogenous glucose (Strasdine 1971), depletion of intracellular P_i, and an increase in specific activity of acid phosphatase. Participation of this enzyme activity in the sporulation process should therefore be considered in any attempts to determine the role of this activity in cell metabolism. Finally, since the enzyme is contained in mature spores and assumes the thermal stability asssociated with these structures, one must also consider the possible role of this enzyme in spore germination.

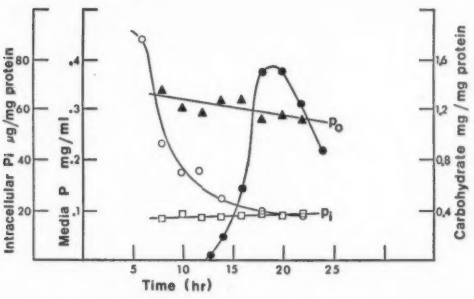

FIG. 4. Phosphorus concentration of cells and media and cellular carbohydrate content during growth of *C. botulinum* type E in trypticase–peptone medium. Intracellular inorganic phosphate, µg/mg protein (○); inorganic (□) and organic (▲) phosphorus of media, µg/ml; carbohydrate mg/mg cell protein (●).

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Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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1588. SAETHER, O. A. 1971. Nomenclature and phylogeny of the genus *Harnischia* (Diptera: Chironomidae). Can. Entomol. 103: 347–362. (4)

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